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314 Albert Street,
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Journal of the Council for Scientific and Industrial Research.

Vol. 17. NOVEMBER, 1944. No. 4

CONTENTS.

Scientific Section.

	PAGE
ETHYLENE CHLOROHYDRIN: A LABORATORY INVESTIGATION OF A CONTINUOUS PROCESS FOR ITS PRODUCTION ON A COMMERCIAL SCALE, by K. E. Murray, B.Sc. (Hons.)	213
STOCK AND SCION INVESTIGATIONS. IV. APPLE MEASLES, by L. A. Thomas, M.Sc.	221
THE QUANTITATIVE SPECTROCHEMICAL ANALYSIS OF AGRICULTURAL SAMPLES, by A. C. Oertel, M.Sc.	225
THE EFFECT OF LIME, PHOSPHATE, AND MOLYBDENUM ON THE GROWTH OF LUCERNE IN DUNTROON LOAM, by N. H. Shaw, B.Agr.Sc., Nancy Barrie, B.Sc.Agr., and E. H. Kipps, B.Sc.	233
DORMANCY AND HARDSEEDEDNESS IN *T. subterraneum*. 4. VARIATION BETWEEN VARIETIES, by K. Loftus Hills, B.Agr.Sc.	242
DORMANCY AND HARDSEEDEDNESS IN *T. subterraneum*. 5. THE EFFECT OF THE CONDITION OF THE SEED COAT UPON EMBRYO DORMANCY, by K. Loftus Hills, B.Agr.Sc.	251
DORMANCY AND HARDSEEDEDNESS IN *T. subterraneum*. 6. THE APPLICATION OF THE RESULTS TO THE PROBLEM OF RE-ESTABLISHMENT IN THE FIELD AND TO SEED TESTING AND MARKETING, by K. Loftus Hills, B.Agr.Sc.	255
PROGRESS OF WORK WITH POTATO STOCKS FREE FROM VIRUS X (FX POTATOES), by J. G. Bald, M.Agr.Sc., Ph.D.	258
DEVELOPMENT OF DIFFERENCES IN YIELD BETWEEN FX AND VIRUS X-INFECTED UP-TO-DATE POTATOES, by J. G. Bald, M.Agr.Sc., Ph.D.	263
THE PERMANENCE OF AUSTRALIAN VERTICAL RETORT CREOSOTE OILS. CORRELATION OF BOILING RANGE WITH LEACHING LOSSES, EVAPORATION LOSSES, AND CHANGES IN COMPOSITION, by David E. Bland, M.Sc.	274
THE EVALUATION OF D.D.T. AS A FUNGICIDE, by D. O. Norris, M.Sc. (Agric.)	289
THE MOISTURE CONTENT OF MEAT EXTRACT. 1. THE NATURE OF MOISTURE CONTENT, by Arthur R. Riddle, A.B., M.S.	291
TESTS FOR QUALITY IN EGG PULP. 2. FURTHER EXPERIMENTS ON THE RESAZURIN REDUCTASE TEST, by W. J. Scott, B.Agr.Sc., and J. M. Gillespie, M.Sc.	299

Notes.

Colonial Research Fellowships	305
Buffalo Fly Investigations	305
Reviews	306
Recent Publications of the Council	307
Forthcoming Publications of the Council	308

(PUBLISHED QUARTERLY)

Journal of the Council for Scientific and Industrial Research.

Vol. 17. NOVEMBER, 1944. No. 4.

Ethylene Chlorohydrin: A Laboratory Investigation of a Continuous Process for its Production on a Commercial Scale.

*By K. E. Murray, B.Sc. (Hons.)**

Summary.

Although much has been written on the formation of ethylene chlorohydrin from ethylene, chlorine, and water, the only descriptions of suitable apparatus for its continuous production are in the patent literature.

The merits of patented continuous processes have been considered and a small plant incorporating features from two of them has been built and successfully operated. Under what seemed to be the optimum conditions the percentage yields of products with reference to the ethylene used were:—Ethylene chlorohydrin 86-88 per cent.; ethylene dichloride 8-9 per cent.; 2: 2′-dichlorodiethyl ether, 1 per cent.

Anhydrous ethylene chlorohydrin has been isolated from the aqueous reaction product in good yield by fractional distillation followed by azeotropic distillation of the concentrated chlorohydrin solution with ethylene dichloride.

A ternary azeotrope of water, ethylene chlorohydrin, and 2: 2′-dichlorodiethyl ether is reported. It has been shown that ethylene dichloride, benzene, and trichloro-ethylene do not form ternary azeotropes with ethylene chlorohydrin and water as reported in the literature.

1. Introduction.

This work was initiated by a request from the Medical Equipment Control Committee to prepare, in the laboratory, ethylene chlorohydrin on a scale adequate for the synthesis of novocaine, sufficient for Australian needs. In order to construct a laboratory apparatus which would allow this request to be fulfilled easily, some study of factors affecting plant design was needed and was considered necessary since it was contemplated later to produce ethylene chlorohydrin on a larger scale.

* An officer of the Division of Industrial Chemistry.

Our knowledge of the reaction between ethylene, chlorine, and water is derived in the first instance from the work of Gomberg[1] who isolated ethylene chlorohydrin and ethylene dichloride as products of the reaction, and visualized the reaction to proceed as follows:—

$$\begin{array}{ccc} H_2O + Cl_2 & \longrightarrow & HOCl + HCl \\ + C_2H_4 & \longleftarrow & + C_2H_4 \\ \swarrow & & \searrow \\ C_2H_4Cl_2 & & C_2H_4(OH)\,Cl \end{array}$$

In order to avoid the formation of dichloride in appreciable amounts, he found it necessary to keep ethylene in excess during the reaction, and to stop when a concentration of 8 per cent. chlorohydrin was reached. The neutralization of the acid formed was undesirable as dichloride formation was favoured more by the metallic chlorides than the free acids.

Later 2:2′-dichlorodiethyl ether was recognized in small amounts as a product of the reaction[2]. Ethyl hypochlorite has been suggested as an intermediate in its formation, but a clear picture of the whole reaction is given by the general mechanism of Ingold (summarized by Robertson *et al.*[3]) for the addition of halogens to ethylene linkages. In the first stage the positive atom of a polarized chlorine molecule attaches itself to the negative carbon atom of a polarized ethylene molecule forming momentarily the chlorethylcarbonium ion, $Cl\,CH_2:CH_2^+$, and neutralization of this positive ion by negative ions present in the solution implies competition for this operation between the chlorethoxyl ($OC_2H_4Cl^-$), hydroxyl and chloride ions giving respectively 2: 2′–dichlorodiethyl ether, ethylene chlorohydrin, and ethylene dichloride.

The findings of Gomberg have been generally confirmed by later workers. In addition, the experimental work of Frahm[4] gives support to the above mechanism, for he was able to reduce the dichloride yield to one-fifth of its former value by the removal of chlorion formed during the reaction by the addition of lead acetate. Bozza and Mamoli[5] have demonstrated that the reaction rate is independent of HOCl concentration over a wide range and is strictly proportional to the rate of absorption of ethylene.

There is little doubt that the batch laboratory process used by Gomberg possesses undesirable features for larger scale operation, in particular, difficulties arising from the corrosive nature of the reaction liquid and the large power input needed for adequate agitation of the liquid. Such disadvantages are reflected in the many patents concerning the reaction which have as their chief object the devising of an efficient continuous process which also involves a satisfactory means of isolating anhydrous chlorohydrin. All these continuous processes propose the introduction of the chlorine and ethylene to the flowing reaction medium with maintenance of a constant chlorohydrin concentration by addition of water to the system at a suitable rate and simultaneous withdrawal

of chlorohydrin solution. They differ, however, in the way contact is made between the gases and the liquid, especially between ethylene and the reaction liquid containing HOCl. The methods used include passing ethylene up a tower countercurrent to the reaction liquid which is trickled over a packing or admitted as a spray, or dispersing the gas in a body of liquid by forcing it through porous diaphragms or, together with the reaction liquid, through jets or orifices.

Although chlorohydrin can often be used as its dilute solution it may be required in either concentrated or anhydrous condition. Gomberg showed that it was impossible to concentrate dilute chlorohydrin solution by distillation, beyond the composition of its azeotrope with water (43·5 per cent. wt. chlorohydrin. B. pt. 97·8° C.[6]). His method of obtaining anhydrous chlorohydrin was tedious and inefficient, and later workers have not published a good laboratory method. Methods reported in the patent literature include solvent extraction with ether, benzene, and ethylene dichloride, or azeotropic distillation with ethylene dichloride or benzene. One patent[7] describes a method in which, after extraction of the ethylene dichloride from the reaction liquid with petroleum ether, the ethylene chlorohydrin is extracted with a 1 : 1 mixture of di-isopropyl ether and n-propanol from which it is then recovered by an azeotropic distillation.

2. Experimental.

(i) *Formation of the Chlorohydrin.*

(*a*) *Choice and design of apparatus.*—An attempt to adapt the batch method of Gomberg to a scale using 18 litres of reaction liquid was unsuccessful owing to the shortcomings previously mentioned, and this led to the consideration of a continuous process. It is not proposed to discuss the relative merits of the patented processes, but they were carefully considered and a small glass plant similar to that described by Ferrero and Valendries[8] was constructed. The essential points of their patent are:—(i) the complete solution of chlorine before contact with ethylene to avoid their combination in the gaseous phase, and (ii) the regulation of the flow rate of the reaction liquid around the cycle to give relatively concentrated solutions of chlorine of at least 15 per cent. of the saturation value (at 20°C.).

Since the reaction liquid is very corrosive the use of a circulating pump by Ferrero and Valendries seemed an undesirable feature of their apparatus, and it proved simpler to omit the pump and follow the device of Britton, Nutting, and Huscher[9], who employ the buoyancy of the gases to circulate the liquid. The final design of the apparatus possessed the following desirable features:—

(i) No moving parts in contact with the reaction liquid.

(ii) Efficient dispersion of the gases by porous candles.

(iii) Ease of construction from easily available and corrosion-resistant material, e.g., earthenware.

The apparatus used was made in one piece from Pyrex and in its final form is illustrated in Fig. 1. Chlorine is introduced into the

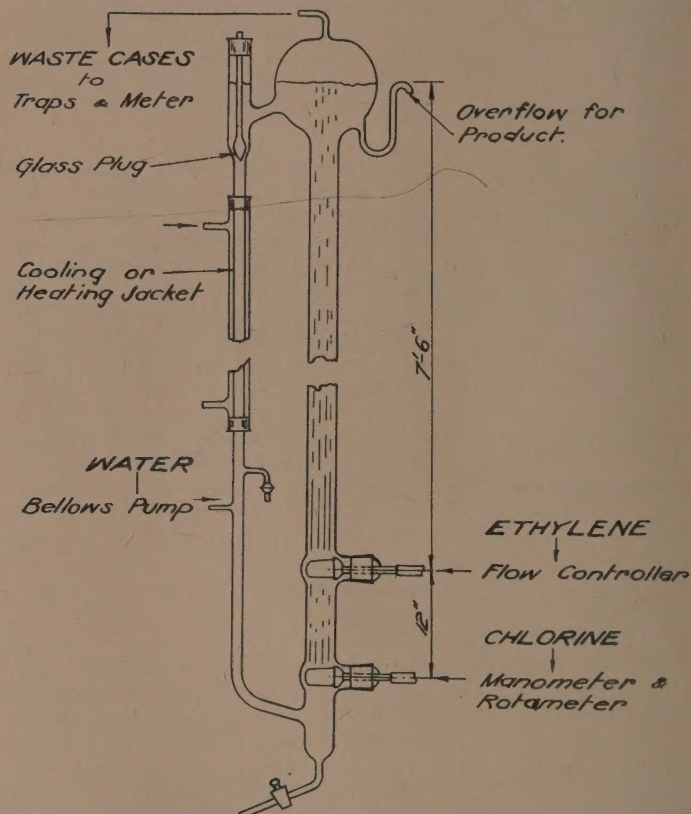

FIG. 1.—Apparatus for the preparation of aqueous ethylene chlorohydrin showing column "A" on right and column "B" on left.

reaction liquid at the base of a vertical column "A" ($1\frac{3}{4}$ in. internal diameter) and is rapidly dissolved. Ethylene is similarly introduced at a point 12 inches higher up and in the form of fine bubbles comes into contact with the chlorine solution flowing up the column. The reaction takes place and the unreacted gases pass out of the liquid at the top of the column where it expands into a 3-litre bulb. A second column "B" ($\frac{5}{8}$ in. internal diameter) carries the liquid free of gases back to the base of "A," and owing to the difference in head between the two columns (calculated as about 5 in. water pressure) caused by the dispersion of gases in "A," the liquid circulates without mechanical aid. The rate of flow was found to be more than adequate even with a relatively small amount of unreacted gases in "A" and was adjusted by the position of a glass plug in column "B." A measurement of the rate was obtained from the time taken for the colour front of some

methylene blue solution, added at the top of column "B," to travel its length. In order to carry out runs at various temperatures, column "B" was fitted with a jacket through which cooling or heating liquid could be passed. During continuous operation the concentration of chlorohydrin was kept constant by the addition of water by a small bellows pump and a compensating overflow of chlorohydrin solution.

The ethylene was prepared by dehydration of ethyl alcohol over a kaolin clay catalyst at 400°C. Its flow rate was kept constant by a simple photoelectric flow-controller and was determined before and after each run by passing it through the apparatus, with chlorine shut off, to a wet test meter. The same meter was used to measure the unreacted gases during a run. The chlorine flow rate was measured by a rotameter and adjusted so that ethylene was always in a slight excess.

Owing to the dependence of reaction rate on the rate of solution of ethylene, it was desirable to obtain a fine dispersion of ethylene in the liquid. The distributors used were kieselguhr filter candles (Doulton, England), 2 in. long, 1 in. diameter, sealed into glass sockets by a non-porous acid-proof cement, the whole being fitted into the apparatus by ground joints. A rapid solution of chlorine and a fine dispersion of ethylene were obtained, but it was noticed that the size of the bubbles was influenced by the presence of the reaction products. Commencing with water in a batch run, it was found that for a short while the bubbles were relatively coarse, after which they became much finer. This effect can hardly be explained by a lowering of surface tension due to the products, as the chlorohydrin concentration would not be more than 0·5 per cent. when this effect became apparent, and the lowering of surface tension at this concentration is not appreciable. Surface tension measurements have been made of a number of synthetic mixtures containing ethylene chlorohydrin up to 8 per cent., HCl in equivalent amount, and ethylene dichloride, and also a number of pure chlorohydrin solutions of similar concentrations. Results show that lowering of surface tension in the region examined depends only on the concentration of chlorohydrin and in almost linear manner. A mixture containing 8 per cent. chlorohydrin, 0·8 per cent. dichloride and molar to HCl, and an 8 per cent. solution of chlorohydrin, both gave a surface tension of 59-60 dynes/cm., a lowering of 13 dynes/cm. A patent[10] has suggested the use of a chlorine-resistant wetting agent to lower the surface tension of the reaction liquid and obtain better dispersion of the gas, but in view of the satisfactory dispersion already obtained, this was considered an unnecessary complication.

(*b*) *Estimation of products: Ethylene chlorohydrin.*—An estimation of the HCl formed during the reaction was taken as equivalent to the chlorohydrin formed. This is not strictly accurate, owing to the small amounts of 2:2′-dichlorodiethyl ether formed, and the yields of chlorohydrin calculated on this basis will be high by twice the percentage yield of ether.

Ethylene dichloride: This was estimated by fractional distillation of the reaction liquid and collected as its heteroazeotrope with water. Much of the dichloride formed is carried off as vapour with the unreacted gases and in runs for which the yields are reported was trapped by cooling them to —20° to —30°C.

2:2′-Dichlorodiethyl ether: Except for several runs at high chlorohydrin concentration, when the ether separated from the reaction liquid along with dichloride, it was not detected in the aqueous product until the completion of the work. This was mainly due to its formation with water and chlorohydrin of a hitherto unreported ternary azeotrope which was found to boil at 97·3°C. (753 mm.), and to have an approximate composition as follows:—

2:2′-Dichlorodiethyl ether	22 per cent.
Water	53 per cent.
Ethylene chlorohydrin	25 per cent.

(*c*) *Effect of temperature on the reaction.*—Several patents have mentioned the use of temperatures up to 80°C.; quantitative runs have been made at three temperatures to determine the effect of temperature on the yields of products. During these runs other conditions were kept constant as follows:—

Concentration of chlorohydrin: 5·1—5·4 per cent. w/v.

Circulation rate of reaction liquid: 260 litres/hr.

Rate of absorption of ethylene (N.T.P.): 58·3—60·8 l/hr.

The yields on the amount of ethylene absorbed are given below:—

TABLE 1.

Temperature.	Yields.	
	Chlorohydrin.*	Dichloride.
Deg. C.	%	%
50 ± 2	87·3	6·0
30 ± 2	89·8	5·3
12–13	88·7	8·2
12–13	87·8	8·8

* These yields include double the yield of dichlorodiethyl ether which was estimated by the fractionation of the combined products of these runs and calculated to be approximately 1 per cent. of the ethylene used.

It may be concluded that temperatures from 12° to 50°C. have no appreciable effect on the yields of the products. The lower yields of dichloride at the higher temperatures are probably due to less complete recovery from the reacted gases.

(*d*) *Effect of the rate of circulation of the reaction liquid on the reaction rate.*—The flow rate of reaction liquid was varied from 50—360 l/hr. without any noticeable change in the reaction rate as judged by the volume of unreacted gases. These rates correspond to concentrations of dissolved chlorine (before contact with the ethylene) of 7 to 50 per cent. of the saturation value (referred to the solubility of chlorine in water at 20°C.).

(*e*) *Effect of chlorohydrin concentration.*—Gomberg and later workers who have used the batch process have agreed that by-products increase considerably when the concentration of chlorohydrin is

increased beyond 8 per cent. In continuous operation, however, the corresponding concentration will be lower, as a continuous process operating at 8 per cent. would be working at a higher concentration than the average of a batch process taken up to 8 per cent. Spence[2] states that under such conditions, the yield from the continuous process was found to be 2 per cent. less than that from the batch process. Yields obtained at 5·1—5·4 per cent. concentration have been given in Table 1 and it is considered undesirable to operate at concentrations higher than this.

In order to obtain a clearer idea than was given by Gomberg of by-product formation at higher chlorohydrin concentrations, a run was carried out under batch conditions in the same apparatus. At intervals the by-products which separated were withdrawn, the HCl and chlorohydrin in the reaction liquid estimated, the HCl by titration and the chlorohydrin by the refractive index of the distillate after fractionation. The reaction temperature was 40°—50°C. and the rate of absorption of ethylene 50-60 l/hr. The yields of ethylene dichloride and 2:2′–dichlorodiethyl ether over four ranges of HCl and chlorohydrin concentrations are tabulated below. The difference between the corresponding concentrations of HCl and chlorohydrin is explained by the extraction of some of the chlorohydrin formed by the separated by-products, as well as by the formation of the ether.

TABLE 2.

HCl Concentration.	Chlorohydrin Concentration.	Yields.	
		Dichloride.	Dichloroether.
		%	%
0–2·4 normal ..	0–1·8 molar	17·9	6·8
2·4–3·5 ,, ...	1·8–2·6 ,,	24·3	8·0
3·5–4·5 ,, ...	2·6–3·1 ,,	35·4	10·1
4·5–5·4 ,, ...	3·1–3·2 ,,	41·9	11·8

(*f*) *Effect of variation of gas flow rates.*—A number of runs were made increasing the ethylene flow rate with corresponding increases in chlorine and water rates. No significant effect on the yield of chlorohydrin was found. At the highest rate, 130 litres/hr. of ethylene (at N.T.P.) was absorbed, corresponding to the formation per hour of 456 g. of chlorohydrin, the yield being 87 per cent. at a concentration of 5·9—6·1 per cent. The percentage of ethylene in the feed absorbed during the run was 90 per cent. The capacity of the distributors was the limiting factor in preventing a further increase in gas flow rates.

(ii) *Concentration and Isolation of Ethylene Chlorohydrin.*

The reaction liquid containing 5-6 per cent. chlorohydrin was fractionated without neutralization, using a glass laboratory column 110 cm. x 2·2 cm. diam., packed with glass helices and fitted with a reflux ratio head. Ethylene dichloride distilled over first as its heteroazeotrope with water (B.Pt. 72°C.), then the ternary azeotrope 2:2′–dichlorodiethyl ether–chlorohydrin–water (B.Pt. 97·3°C.), and

finally the chlorohydrin–water azeotrope (B.Pt. 97·8°C.). Solutions of chlorohydrin of 35—40 per cent. w/v could be easily obtained. In order to obtain pure chlorohydrin the fraction containing the dichlorodiethyl ether was collected separately, as it forms an azeotrope with chlorohydrin (B.Pt. 128·2°C.[11]) which would be difficult to separate from anhydrous chlorohydrin (B.Pt. 128·6°C.) by distillation. Kireev, Kaplan, and Zlobin[6] have obtained vapour equilibria data for the system ethylene chlorohydrin–water, which permit the design of a column for the concentration of the chlorohydrin in a continuous manner. The value of this calculation is limited by the presence of ethylene dichloride and dichlorodiethyl ether in the reaction product.

Anhydrous chlorohydrin has been recovered from 35–40 per cent. w/v solutions in good yield, following the method described by Ernst and Kaufler[12]. Batches of 1½ litres of the concentrated chlorohydrin with 300 ml. of ethylene dichloride were distilled, the water being carried off as its heteroazeotrope with ethylene dichloride. The vapours were passed through a fractionating column, condensed, when the condensate separated into layers, the lower layer, the dichloride, being continuously returned to the flask. When water was completely removed (after 5—7 hours) the mixture of dichloride and chlorohydrin could be easily separated by fractionation. Recoveries of 85—90 per cent. of the theoretical have been obtained with less than 1 per cent. loss by hydrolysis. The balance of chlorohydrin could be mostly accounted for in the aqueous layer of the distillate. It was found that loss in this way could be diminished by better fractionation, but the process would then take longer. For commercial production, continuous operation in a column as suggested by Ernst and Kaufler would no doubt be feasible and more efficient.*

In their patent, Ernst and Kaufler have described three ternary azeotropes which chlorohydrin and water form with benzene, ethylene dichloride, and trichlorethylene, containing relatively small amounts of chlorohydrin (below 1 per cent. by wt.). These are reported in Beilstein (Bd. I., Zweites Erg. Werk, p. 334) as ternary azeotropes of ethylene chlorohydrin but investigation has shown them to be fictitious. A mixture of benzene, chlorohydrin, and water of the composition assigned by the patent to the azeotropic mixture was fractionated through a glass column packed with helices using an Auschutz thermometer checked against an N.P.L. standard. The bulk of the distillate was found to possess an identical boiling point with the heteroazeotrope of benzene and water when this was distilled through the same column. No chlorohydrin was present, for the refractive indices of the water layers of the two distillates were identical. Similar results were found with the mixtures of chlorohydrin—ethylene dichloride—water and chlorohydrin—trichlorethylene—water.

Attempts have been made to work out in the laboratory a suitable simple solvent extraction recovery process, but without any notable success.

* It should be possible to combine the distillation of the azeotropic mixture of chlorohydrin and water with the subsequent azeotropic dehydration of the chlorohydrin with ethylene dichloride, and this process is claimed in the Australian Patent No. 28465/30, which describes the "Fourth Technique" of Usines de Melle for the dehydration of ethanol.

3. Acknowledgment.

The writer gratefully acknowledges the advice and assistance of Dr. H. H. Hatt during this work.

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It seemed undesirable to append a full bibliography. Many of the patents concerning the reaction are abstracted in Ellis, "The Chemistry of Petroleum Derivatives," Vol. I. (1934), p. 488 *et seq.*, Vol. II. (1937), p. 515 *et seq.*

Stock and Scion Investigations.

IV. Apple Measles.

*By L. A. Thomas, M.Sc.**

Summary.

The symptoms of a disorder known as apple measles are described. Superficial cork and dieback were associated with measles, and all these disorders were controlled by borax-containing sprays.

1. Introduction.

During the past five years a disorder appeared on young Jonathan and Delicious apple trees and on Williams pear trees at the Council's Field Station at Stanthorpe, Queensland. Most of the affected trees were in nursery rootstock trials where the planting distance is usually 4½ feet by 5 feet; but several Jonathan trees planted 20 feet apart have shown the same symptoms at four years of age.

* Research officer of the Division of Plant Industry stationed at Stanthorpe.

2. Symptoms on Apple Trees.

(i) *Bark and Wood.*

Flecks or small patches, purple-brown or bronze in colour, appear on the bark of young trees, mainly on the northern sides of the shoots. Raised pimples about 1/16 inch in diameter appear later; beneath these the bark tissues are dead. Often a pink colouration develops in the cambial region beneath the pimples, this usually occurring towards the onset of the dormant period. About these pimples the bark dies and cracks in a roughly circular fashion; these cracked areas may become confluent so that patches of bark die and expose the wood beneath.

The cracking of the bark is often more noticeable on the shaded southern side of the tree in the earlier stages. Dieback of terminal growths occurs and is noticed particularly in late summer or autumn. Sucker growths from old wood appear after the bark symptoms are pronounced. The bark symptoms are illustrated in Plates 1, 2, 3, 4.

No leaf symptoms have been observed, but defoliation of the trees occurs earlier than with those in normal growth.

(ii) *Fruit.*

Fruits of the apple variety Jonathan showed typical superficial cork symptoms; young fruits up to ¾ inch in diameter showed russeting and deep cracks; older fruits were russeted, slightly malformed, and "pebbly." Mature Jonathan fruits of good quality had shallower calyx basins than normal fruit. Fruits of the variety Delicious were paler and rougher-skinned than normal, and would be described as having a "poor finish." The five "points" at the calyx end of the fruit were not prominent and often absent.

3. Symptoms on Pear Trees.

Several Williams pear trees developed the bronzed pimpled bark symptoms, dieback of terminal growth, and early defoliation in the autumn as observed in apple trees. No fruits had been borne on these three-year-old trees.

4. Material and Treatment.

(i) *Apple Trees.*

In three apple nursery rootstock trials, A, C, and D, about 160 trees showed definite symptoms of the disorder. Trial A consisted of Jonathan trees with Granny Smith guard rows; trial C of Jonathan trees with Granny Smith and Delicious guard rows. Both these trials were planted on virgin ground and had received applications of copper and zinc as a safeguard against summer dieback and little leaf. Trial D consisted of Delicious apple trees with Gravenstein and Jonathan guard rows, and was planted on ground that had grown a nursery of apple trees twelve months previously. As the trees in this trial exhibited the most severe symptoms, with two trees dead after two years' growth, it was abandoned as a stock trial and used for a control plot.

Trees in nursery trials A and C were sprayed with borax solutions incorporated in the calyx and first cover sprays each year; in 1941 at the rate of 1 lb. per 100 gallons spray mixture, and in 1942 and 1943 at the rate of 5 lb. per 100 gallons spray mixture. The five Jonathan trees planted 20 feet apart had similar spray treatments plus farmyard manure dug in the soil. Trees in trial D were assessed for severity of symptoms during the dormant season of 1941, and rated 0 to 5 accordingly. Twelve plots, each of four trees, were chosen to receive the following treatments:—

(1) Farmyard manure at the rate of 20 tons per acre applied 5th October, 1941.

(2) Borax spray at the rate of 2 lb. per 100 gallons, approximately $\frac{1}{3}$ gallon per tree, applied 15th October, 1941, and 12th November, 1941.

(3) Borax spray at the above rate on 15th October, 1941, and 12th November, 1941; Bordeaux mixture (Bordinette) at 10 lb. per 100 gallons on 12th November, 1941, $\frac{1}{3}$ gallon per tree; zinc sulphate, 10 lb. per 100 gallons, plus hydrated lime 5 lb. per 100 gallons, on 12th November, 1941, $\frac{1}{3}$ gallon per tree.

A dressing of $\frac{1}{4}$ lb. per tree of a fertilizer mixture consisting of two parts sulphate of ammonia, two parts superphosphate and one part muriate of potash was applied on 2nd October, 1941.

(ii) *Pear Trees.*

Williams pear trees were planted in an old apple tree nursery after the ground had lain idle for two years. These received borax spray 5 lb. per 100 gallons and farmyard manure at the rate of 10 tons per acre in 1943.

5. Results.

For nursery trials A and C, the results are in the form of observations. These trees, over the three-year period of treatment, lost their pimpled condition, generally in the second year of treatment; new growth remained healthy and dead areas on the twigs callused over. The purplish bronze colour of the older wood faded or disappeared, the cracked bark sloughed off on the vigorous trees and generally became less evident. Only occasional small fruits showed superficial cork, and two crops of normal fruit have been produced. Both the Jonathan trees planted 20 feet apart and the Williams pear trees recovered during the growing season in which the treatments were applied.

In trial D, where the treatments were applied for one year only, considerable improvement in tree health was noticed during the growing season of 1941-42. The ratings for severity of symptoms for each tree were obtained during the dormant season of 1942, and the information for the various treatments is presented in Table 1.

TABLE 1.—ESTIMATED SEVERITY OF SYMPTOMS ON DORMANT TREES.

Treatment.	Plot 1.	Plot 2.	Plot 3.	Total.
Borax spray ..	Before 3222 After 1001	3122 1012	1112 1011	22 9
Borax, copper, and zinc sprays	Before 1233 After 0221	2111 1100	2123 1011	22 10
Farmyard manure ..	Before 4332 After 4121	2221 2220	3222 2111	28 19
No treatment ..	Before 2121 After 3222	3322 5553	2111 2222	21 35

6. Discussion.

The bark symptoms as described above, agree with those described as apple "measles" by Young and Winter (1937) for Red Delicious apple trees. They believe this disorder, which is associated with a suckering habit, is due to boron deficiency. Burrell (1937) lists several symptoms, including drouth spot (superficial cork), internal cork, dieback, and rosette as symptoms of boron deficiency. The development of these closely related disorders depends upon the season and the variety of fruit.

In New South Wales (Anon., 1944) it has been found that different types of symptoms of leaves, twigs, and fruit occur depending on climatic and soil conditions, or on the time when boron becomes deficient.

Hence, it is concluded that the combination of symptoms of boron deficiency given above, need not necessarily be constantly shown, even in this district.

7. Acknowledgments.

My thanks are due to R. B. Morwood, M.Sc., of the Queensland Department of Agriculture and Stock, who diagnosed the bark symptoms as apple measles.

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The Quantitative Spectrochemical Analysis of Agricultural Samples.

*By A. C. Oertel, M.Sc.**

Summary.

The elementary theory is given for a quantitative method of analysis based on the addition, to portion of the sample, of known amounts of the elements to be estimated. The assumption is made that, when all other variables are controlled, the total radiant energy emitted by an element is proportional to some power of the mass of that element entering the source of excitation. The method is particularly suitable for agricultural samples because it avoids the necessity for the wide range of synthetic standards required by other methods.

1. Introduction.

One of the most obvious methods of quantitative spectrochemical analysis, the method in which the concentration of an element is determined by measuring the increase in intensity of one of its spectral lines produced by the addition of a known amount of the element to a portion of the sample, appears to have received little attention. Workers who have investigated the method (6, 8, 10) all seem to be under the impression that it has not been tested, or, at the most, not thoroughly tested. Hasler (10) suggests it as "a powerful rough general method of quantitative analysis with a very wide field of application since it may be used where the preparation of suitable standards would be tedious and uncertain". An example given to indicate the degree of accuracy that may be expected shows a positive error of about 30 per cent. in the estimation of a concentration of 35 parts per million. Dewar (6, 1) tested the method on synthetic plant ashes, and obtained errors nearly all of which were positive and the majority in the range 15 per cent. to 50 per cent. in estimating concentrations in the range 30 to 300 parts per million. Foster and Horton (8) used the same simple assumption as that used by Hasler and by Dewar, namely that the intensity of a spectral line varied directly as the mass of the element (or as its concentration in samples of constant total mass), and they showed that it was valid for boron over the small range of concentrations of interest (up to about 10 parts per million). Results obtained by them rarely varied as much as 10 per cent. from the mean of a long series. The tests of the validity of the assumption also revealed that it is not valid at higher concentrations, when the intensity does not increase as rapidly as the concentration. This may account for the large positive errors obtained by the other workers.

It is believed that results at least as accurate as those of Foster and Horton can be obtained in all cases if measurements made on the spectrograms are interpreted on the basis of a more general theoretical assumption.

* An officer of the Division of Soils.

2. Theory of Method.

It is sometimes assumed that, in certain limited ranges of concentration at least, the intensity of the radiation emitted by an element in an excited sample is directly proportional to the concentration of the element, or to put it in another way, the total amount of energy emitted by an element in an excited sample is directly proportional to the mass of the element in the sample. The "total energy method" of Slavin (16) is based on that assumption, and he found that the relation was sufficiently accurate for practical purposes, held for all concentrations, and was independent of the nature of the sample, at least for the limited number of samples he analysed by the method. The great majority of workers have found, however, that the relationship between intensity and concentration (or total energy and mass of element) is not, in general, linear, and that the relationship is affected by the presence of other elements in the sample (e.g., 3, 7, 11, 13, 17). It seems obvious that the excitation of a sample containing many elements by an electric arc or spark is a very complex affair, and will depend with arc excitation, for example, not only on the general composition of the sample but also on the electrodes used to hold the sample, the current in the arc, the length of the arc, and the polarity of the sample, to mention a few factors.

Probably one of the simplest assumptions that can be made with the present knowledge, and one which is supported by the experience of most spectrochemists, is that

$$E_\lambda = k\, m^n, \qquad (1)$$

in which E_λ is the total amount of radiation of wavelength λ which is emitted by the element and leaves the source of excitation (that is, that part of the radiation in which the spectrochemist is primarily interested); m is the total mass of the element (hereinafter called the test element) which enters the source of excitation; and k and n are constants over at least a small range of concentrations of the test element. The values of the constants k and n will, in general, depend on—

(i) the wavelength of the radiation;

(ii) the means and method of exciting the sample;

(iii) the concentration of the test element;

(iv) the properties of the atoms of the test element;

(v) the properties of the test element;

(vi) the properties of the chemical compound of the test element present in the original sample;

(vii) the factors (iii), (iv), (v), and (vi) for all other elements present in the original sample; and

(viii) the properties and composition of the original sample taken as a whole.

The average intensity of the radiation over the exposed area of the slit of the spectrograph during an exposure is given by

$$E_\lambda l/4\pi d^2 t,$$

in which t is the duration of the exposure, d is the distance of the source from the slit, and l is a factor introduced by a condensing lens which is unity when no such lens is used. Putting I_λ for the average intensity of radiation of wavelength λ at the slit, it follows from equation (1) that

$$I_\lambda = klC^n M^n/4\pi d^2 t, \tag{2}$$

in which C is the concentration of the test element, and M is the total mass of the original sample, complete volatilization being assumed. Equation (2) may be written

$$I_\lambda = aC^n, \tag{3}$$

in which the constant a is given by

$$a = klM^n/4\pi d^2 t. \tag{4}$$

When a set of practically identical samples is to be analysed, one may probably assume with reasonable safety that the amount of test element entering the source of excitation in a given period is proportional to the amount of the element originally present in the sample on the electrodes, provided that there is always excess sample at the end of the selected period. Then

$$m = pCM,$$

in which p is a constant, and the value of a is given by

$$a = klp^n M^n/4\pi d^2 t. \tag{5}$$

When the sample is introduced into the source of excitation as a solution which is caused to flow at a steady rate through the source, the mass of the sample in the source is constant throughout the exposure. The sample is also quite homogeneous, and one has a most favorable set of conditions for accurate results.

Assume now that we have a sample in which the test element is present at a concentration less than one part in one thousand, and that an "internal standard" element (which may or may not have been added by the analyst) is present in the sample. From equation (3), dropping the subscript λ, and using the subscript T for the test element and S for the internal standard element,

$$I_T = a_T C_T^{n_T}, \text{ and } I_S = a_S C_S^{n_S};$$

from which

$$\log (I_T/I_S) = \log (a_T/a_S) + n_T \log C_T - n_S \log C_S.$$

The value of C_S is necessarily constant, and assuming that the factors which may affect the values of a_T/a_S, n_T, and n_S are all sufficiently constant,

$$\log (I_T/I_S) = n_T \log C_T + A, \tag{6}$$

in which A is a constant. It is well known that when the logarithm of the ratio of the intensities of "test" and "internal standard" lines is plotted

against the logarithm of the concentration of the test element, the calibration curve is often straight over a large range of concentrations, and is probably always straight over at least a small range. One may, therefore, assume that equation (1) is a close approach to the true equation over at least small ranges of concentration, and that the assumed constants are, in practice, sufficiently constant. Of course, equation (3) may be derived by using equation (6) as the starting point (e.g., 2, 4, 13), but then the significance of the constants is not so apparent.

The value of the constant A is given by

$$A = \log (a_T/a_S) - n_S \log C_S.$$

If we assume complete volatilization of the sample, and expand a_T/a_S in accordance with equation (4),

$$a_T/a_S = M^{n_T - n_S} k_T/k_S.$$

It is, therefore, independent of the time of exposure and the "set-up" factor within, of course, the limits imposed by the assumptions made. In the case of an ideal internal standard element the mass of the sample may be varied, because in that case n_T equals n_S. In practice, when the internal standard is selected so that n_T and n_S are as nearly equal as practicable, small variations in the mass M will be immaterial. If we assume only partial volatilization of the sample*, and expand a_T/a_S in accordance with equation (5),

$$a_T/a_S = M^{n_T - n_S} k_T p_T^{n_T}/k_S p_S^{n_S};$$

from which similar conclusions may be drawn.

If we use, say, units of parts per million to measure concentrations, and put $C_T = 1$, we get another aspect of the significance of the constant A, for then its value is given by, from equation (6),

$$A = \left\{\log (I_T/I_S)\right\}_{C_T = 1};$$

and equation (6) becomes†

$$\log (I_T/I_S) = n_T \log C_T + \left\{\log (I_T/I_S)\right\}_{C_T = 1}. \qquad (7)$$

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* Methods of analysis in which only partial volatilization of the sample occurs have often been condemned, except for the analyses of metals and alloys when the electrodes are made from the samples to be analysed. That case is an extreme one of partial volatilization of the sample, and profitable advantage is often taken of the fractional volatilization which always occurs. For any type of sample it seems less reasonable to assume, as is sometimes done, that equal amounts of the test element volatilizing at very different rates from different samples, each of which is subjected to excitation until it is completely volatilized, will emit equal amounts of spectrochemically useful radiation, than to assume (as is done here) that equal proportions of the test element will volatilize in equal periods from almost identical samples containing comparable amounts of the test element and excited under nearly as practicable identical conditions, provided always that there is an excess of sample at the end of the period.

Probably one of the best methods for controlling exposures, for similar samples, would be to expose for a selected number of ampere-seconds. A device similar to the usual kilowatt-hour meter could be used to operate an electromagnetic shutter placed in front of the slit.

† The constant required to allow for selection of units of concentration is omitted; it cancels in the final equations (8), (9) and (10).

Assume now that we have two samples which were originally halves of one sample, that the concentration of the test element was increased in one half, by the addition of a measured amount of the element, from C_1 to C_2, and that C_1 and C_2 are comparable. We may then assume that all the constants will be identical for the two samples. Use equation (7) altering the notation as follows :—

$$L_0 = (I_T/I_S) \text{ when } C_T = 1 ;$$
$$L_1 = (I_T/I_S) \text{ when } C_T = C_1 ;$$
$$L_2 = (I_T/I_S) \text{ when } C_T = C_2 ;$$
$$C_1 = (C_T = C_1) ;$$
$$C_2 = (C_T = C_2) ;$$

and $n = n_T$.

Then
$$\log L_1 = n \log C_1 + \log L_0 ;$$
$$\log L_2 = n \log C_2 + \log L_0 ;$$

and
$$\log (L_2/L_1) = n \log (C_2/C_1). \qquad (8)$$

When n is unity we get the simple equation $L_2/L_1 = C_2/C_1$ used by Foster and Horton (8) ; and C_1 is easily calculated. In general, however, n is not known. If the original sample were divided into thirds, and additions of the test element made to two of the parts, we would get in addition to equation (8),

$$\log (L_3/L_1) = n \log (C_3/C_1). \qquad (9)$$

From equations (8) and (9)

$$\log (L_3/L_1)/\log (L_2/L_1) = \log (C_3/C_1)/\log (C_2/C_1). \qquad (10)$$

Solution of equation (10) gives a value for C_1 ; and values of n are obtained from equations (8) and (9). (Equation (10) is easily solved by preparing a table of values of $\log (C_3/C_1)/\log (C_2/C_1)$ for graduated values of C_1 and fixed values of the additions made to give C_2 and C_3. Such a table reveals that there are optimum values for the additions for a given value of C_1. This, and other interesting properties of equation (10), may also be discovered mathematically.)

One of the most satisfactory methods of measuring the required ratio of the intensities of the test and internal standard lines involves the use of the rotating stepped sector and a photographic plate. The characteristic curve of a stepped sector, obtained by plotting the density of the photographic image against the logarithm of the time of exposure, is of a form similar to that of a true blackening curve ; and the equation

$$D = \gamma_s \log It_s - i \qquad (11)$$

represents sufficiently accurately the straight portion of the curve. In the equation, D is the photographic density of the image, I is the intensity of illumination, which is constant ; t_s is the time of exposure, as controlled by the stepped sector ; γ_s is the slope of the straight portion of the curve ; and i is the inertia of the plate. In practice, the intensity is not constant throughout an exposure, but it will, on the average, be the same over the stepped image at any instant, unless the fluctuations in intensity are steady, and at a rate which is some multiple of the speed of rotation of the sector.

Consider the stepped photographic images of a test and an internal standard line, the difference in wavelength being such that γ_s and i may be assumed to be the same for both images. Select two adjacent steps on the test image, corresponding to steps n_T and $(n_T + 1)$, and one on the internal standard image, corresponding to step n_S; and such that the density of the step n_S is intermediate between the densities of the steps n_T and $(n_T + 1)$. Then

$$D_{n_T} > D_{n_S} > D_{(n_T + 1)}. \qquad (12)$$

The steps are numbered so that n is zero for the step of full uninterrupted exposure, and the exposure of a step n

$$t_n = T/R^n,$$

in which T is the full exposure, and R is the ratio of the sector. Substituting in equation (11), and re-arranging,

$$D_{n_T} = \gamma_s \log I_T + \gamma_s \log T - \gamma_s n_T \log R - i; \qquad (13)$$

$$D_{n_S} = \gamma_s \log I + \gamma_s \log T - \gamma_s n_S \log R - i; \qquad (14)$$

and $$D_{(n_T + 1)} = \gamma_s \log I_T + \gamma_s \log T - \gamma_s (n_T + 1) \log R - i. \qquad (15)$$

From equations (13) and (14), subtracting and re-arranging,

$$\log (I_T/I_S) = (D_{n_T} - D_{n_S})/\gamma_s + (n_T - n_S) \log R. \qquad (16)$$

Similarly from equations (13) and (15),

$$\gamma_s = (D_{n_T} - D_{(n_T + 1)})/\log R.$$

Substituting the value for γ_s in equation (16),

$$\log (I_T/I_S) = [(n_T - n_S) + (D_{n_T} - D_{n_S})/(D_{n_T} - D_{(n_T + 1)})] \log R. \qquad (17)$$

(The same result may be obtained in other ways (e.g., 2).)

The first term in the square brackets is a whole number which may be positive or negative, and is obtained by a simple count of steps; the second term is, as a result of relation (12), necessarily a positive proper fraction (or, of course, zero), and is obtained from the density measurements. Sectors are usually made so that $\log R$ is a round number, e.g., 0·2 or 0·3. Constants of the plate are not present as such in equation (17), and, provided that the constants retain the same values for both test and internal standard images, some latitude in the development of the plate may be allowed, and spectrograms to be compared need not necessarily be recorded on the same plate.

3. Discussion.

The most generally useful methods of spectrochemical analysis are based on the internal standard method described by Gerlach and Schweitzer (9). The concentration of a minor component of the sample is correlated with the ratio of the intensities of two lines in the spectrogram of the sample. One of these lines is emitted by the minor component: the other may be a line emitted by the main component, or one of the major components, of the sample, or it may be a line emitted by an element—the internal standard element—added to the sample by the analyst. None of the major

components of a biological ash has a spectrum sufficiently complex for the use of its lines as internal standard lines, and, consequently, an internal standard element is usually added. Of the major components of a soil, iron may be used as the internal standard element when present at not too low a concentration (5, 12). The addition of an internal standard element to a soil sample is not a simple matter because nearly all the common elements, except perhaps cadmium, are usually present in the sample in detectable amounts.

Because the concentrations of the major components of biological ashes and, especially, soils may vary over a great range, and the effect of many of the major components on the ratio of the intensities of the test and internal standard lines may vary with the concentration of the major component, a large number of synthetic standard samples is necessary if an accurate analysis is required of any biological ash or soil that may be encountered. In practice the reproduction of a soil sample is out of the question ; one has to be satisfied with a rough approximation. The accurate reproduction of a biological ash as obtained by the dry-ashing method is also impracticable. When a biological material is dry-ashed, an unknown fraction of each of the many minor metallic components may be in the ash as a silicate (14, 15). The reproduction of the liquid extract of a soil sample, or of a biological ash as obtained by a wet method of "ashing", is, of course, practicable. However, one would require a very great number of standard samples, and a great deal of preliminary work would be necessary to determine the effect of the variation in concentration of each major component on the ratio of the intensities of each pair of test and internal standard lines. Not only would this have to be done, for each major component, for each minor component to be determined, but the effect of varying amounts of the other major components, as the concentration of the one in question is varied, on the effect in question would also have to be determined. For example, the effect of a variation in the concentration of iron on the ratio of intensities of tin and cadmium (internal standard) lines may vary with variations in the concentration of calcium, or manganese, or sodium. Until one had completed this investigation, one could not feel confident that a sample on hand was sufficiently like one of the available standard samples to allow the obtaining of accurate, reliable results.

When the method of additions is used, the standards for any one sample are parts of that sample. If the sample is some biological material, the additions are made to the original material. One may assume with some confidence that, at the end of a process of thorough sulphation and dry-ashing, the added element and that originally present are in the ash in sufficiently similar states, especially as the two amounts are quite comparable. Samples of soil may present some difficulties. If one adds the element as a relatively involatile compound, such as oxide or sulphate, and if the sample of soil is rapidly fused in the arc, one may assume that at least the major portion of the added element will be present in the hot, fused sample in the same state as that part originally present. With wet-ashed biological material and liquid extracts of soils there are no such difficulties.

The method is not so long as may appear. When a number of quite similar samples is encountered, one need do "duplicates" of only a few, carefully selected, and "triplicates" of a few of these. These "duplicates" and "triplicates" then serve as standard samples for the remainder of the batch. These "standard" samples are much more like the "test" samples than the most carefully prepared synthetic standards could be,

especially in the case of soil samples. With the occasional very unusual sample, the time required to do it in "triplicate" is very much less than would be consumed in preparation of synthetic standard samples, especially when analyses are to be made for a number of minor components. And, of course, any suitable element present in the sample, not being a "test" element, may be used as the internal standard for the method of additions.

A useful feature of the method is that the intra-comparison of the "standards" and the inter-comparison of "samples" and "standards" may be used to increase the probable accuracy of individual determinations. The estimation of the concentration of an element when the concentration is so low that the element is just detected, or just escapes detection, in the initial spectrogram used to determine the amounts to be added, involves no additional special work.

4. Conclusion.

It is hoped, at a future date, to publish the results of a critical test of the method as applied to a variety of agricultural samples. This preliminary account may be of interest to other spectrochemists who analyse large numbers of samples of very variable composition. Work so far done has shown that results at least as accurate as those given by the more usual methods are obtained under similar conditions. In a laboratory where the samples to be analysed do not have standardized compositions, the method obviates the almost continuous necessity of preparing synthetic standard samples which would otherwise be the case. One may change the type of photographic plate, the processing solutions and conditions, the conditions under which the samples are excited; in short, one may select freely the ways and means of obtaining the desired results which are most suitable and convenient for the sample on hand, without incurring the necessity of laboriously re-calibrating a method for new conditions.

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The Effect of Lime, Phosphate and Molybdenum on the Growth of Lucerne in Duntroon Loam.

By N. H. Shaw, B Agr.Sc., Nancy Barrie, B.Sc.Agr.*, and E. H. Kipps, B.Sc.**

Summary.

Lucerne growing on Duntroon loam has shown marked deficiency symptoms and has made poor growth. A pot experiment has indicated that healthy, vigorous plants are obtained by the addition of lime, and that yield is increased substantially.

The application of molybdenum gave a slight significant increase in yield in the absence of lime, but only after a number of harvests had been removed, and gave no increase in the presence of lime.

Phosphate gave a response only in the presence of lime.

Data showing the effect on the chemical composition of the plant of applications of lime, molybdenum, and phosphate are presented.

1. Introduction.

Unsatisfactory growth of lucerne has been observed in certain areas on the Duntroon–Dickson experiment station. Plants establish well, but subsequent growth is poor. Even good rains fail to promote healthy growth. New shoots appear healthy until about six inches high, at which stage the leaflets begin to turn yellow, wither, and finally drop off, leaving the petiole attached to the stem. The yellowing proceeds from the base of the stem upward until only the terminal leaves remain. The yellow leaflets are generally partially folded, and often show an accumulation of purple pigments in the vascular tissues. Few flowers are produced by the affected plants, and no new growth is made until the plants are cut back. Plants growing on slightly higher ground are usually most severely affected.

Several preliminary field trials were commenced in the autumn and spring of 1941 to determine whether topdressing with fertilizers containing lime, superphosphate, trace elements, and miscellaneous materials, such as wood ashes, would improve the growth of lucerne.

In the spring of 1941, plots selected as outstanding by several independent observers were those which had received applications of molybdenum. Another stand appeared to have benefited from the addition of lime, but superphosphate gave variable responses.

A pot experiment was conducted to examine the effects of these fertilizers in more detail, using soil taken from the top six inches of an area at Duntroon.

2. Experimental Procedure.

The experiment was designed to test the effect of varying rates and combinations of lime, superphosphate, and molybdenum on the health, productivity, and chemical composition of lucerne growing in Duntroon red loam. The fertilizers were applied at the following

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rates, in all combinations, making 27 treatments: Calcium hydroxide equivalent to 0, 1 and 2 tons of $Ca(OH)_2$ per acre; monocalcium phosphate equivalent to 0, 2, and 5 cwt. of superphosphate per acre; sodium molybdate equivalent to 0, ½, and 2 lb. of molybdenum per acre. Enamel pots (9 in. diameter) containing 14·3 kilograms of Duntroon soil were used. There were four replications of each treatment arranged in randomized blocks. The lime and phosphate fertilizers were mixed with the top 3 kilograms of soil, the molybdenum salt being added in solution later. Lucerne was sown in the pots on November 25, 1941, and the seedlings thinned to leave five plants per pot.

The pots were weighed once a week, and sufficient rain water added on each occasion to bring the soil to a moisture content of 22 per cent. During the summer, when transpiration was high, water was added three or four times between weekly weighings, the amount of water added being determined by the loss during the previous week.

Observations were made on the health and vigour of the lucerne. During the summer, the plants were harvested at the early flowering stage and in the winter, when the plants failed to flower, at intervals of approximately eight weeks. The pots were maintained until 4.10.43, a total of twelve harvests being made. The material harvested was dried at 98°C., weighed, and ground for chemical analysis.

3. Results.

(i) *Effect of Fertilizers on Health.*

Plants growing in soil to which no fertilizer was added began to show symptoms prior to the first harvest similar to those observed in the field. These symptoms became more marked after subsequent harvests. Older leaves began to yellow at the tips of the leaflets, and the margins round the yellow areas turned brown and became dry and papery. More rarely the yellowing spread from spots in the tissues between the veins over the whole leaflet. The plants were stunted, formed few flowers, and had a generally yellow or pale-green appearance. The yellow leaflets tended to wither and fall off. After being cut back, the plants made apparently normal growth until about 4—6 inches high.

All limed pots produced healthy and vigorous plants. Plants which received molybdenum, but no lime, made better growth than the unfertilized plants, but were generally pale or yellow in colour, though they did not show the severe symptoms of stunting and leaf fall.

The addition of phosphate alone made no improvement in the health of the plants.

During November, 1942, the plants were attacked by downy mildew. Of the 36 unlimed pots, 33 were affected, compared with only 8 of the 72 limed pots.

(ii) *Effect of Fertilizers on Yield.*

Yields of dry matter for each harvest are given in Table 1 (A and B), and in Fig. 1. As there is no interaction between molybdenum and phosphate, the 3 x 3 x 3 factorial table of treatments has been condensed

for purposes of presentation to two 3 x 3 tables, the first (A) showing the yields for different levels of phosphate at different levels of lime (molybdenum levels pooled), and the second (B) showing the yields for different levels of molybdenum at different levels of lime (phosphate levels pooled).

TABLE 1.—YIELD OF LUCERNE IN GRAMS PER 12 POTS.

—	Date of Harvest.	(A) Monocalcium Phosphate per Acre.			(B) Molybdenum per Acre.			Total
		Nil.	2 cwt.	5 cwt.	Nil.	½ lb.	2 lb.	
Lime Nil.	* 9.2.42	149	147	130	134	145	147	426
	* 30.3.42	57	76	68	68	67	66	201
	27.5.42	45	44	57	42	54	50	146
	14.8.42	66	68	69	56	80	67	203
	* 6.11.42	220	235	231	146	284	256	686
	*18.12.42	98	104	87	52	104	132	288
	* 2.1.43	85	93	93	56	100	114	270
	* 1.3.43	76	93	84	52	93	109	254
	26.4.43	150	183	182	98	202	215	515
	21.6.43	69	83	95	48	89	111	248
	23.8.43	67	70	75	47	73	93	213
	4.10.43	59	62	61	47	63	72	182
	..	1,141	1,258	1,232	846	1,354	1,432	..
Lime—1 ton/acre.	* 9.2.42	165	180	154	162	167	170	499
	* 30.3.42	170	196	164	177	177	177	531
	27.5.42	179	176	234	197	201	191	589
	14.8.42	133	132	162	140	156	131	427
	* 6.11.42	333	438	519	425	540	416	1,291
	*18.12.42	185	198	239	206	212	205	623
	* 22.1.43	117	134	139	129	137	124	390
	* 1.3.43	95	120	145	121	121	119	361
	* 26.4.43	189	260	297	263	236	247	746
	21.6.43	106	119	156	136	119	127	382
	23.8.43	92	107	129	113	108	107	328
	4.10.43	71	83	97	91	84	75	250
	..	1,835	2,143	2,435	2,160	2,168	2,089	..
Lime—2 tons/acre.	* 9.2.42	156	186	164	162	177	167	506
	* 30.3.42	181	189	192	175	202	185	562
	27.5.42	151	165	162	158	161	159	478
	14.8.42	118	142	144	130	124	149	403
	* 6.11.42	335	434	521	430	434	425	1,289
	*18.12.42	219	233	256	231	238	240	709
	* 22.1.43	129	139	143	136	137	138	411
	* 1.3.43	117	133	145	129	129	137	395
	* 26.4.43	253	287	323	294	288	282	864
	21.6.43	129	172	181	148	177	157	482
	23.8.43	109	137	144	124	137	130	391
	4.10.43	89	103	103	97	106	93	296
	..	1,986	2,320	2,478	2,214	2,310	2,262	..

* Plants were harvested at the early flowering period on these dates.

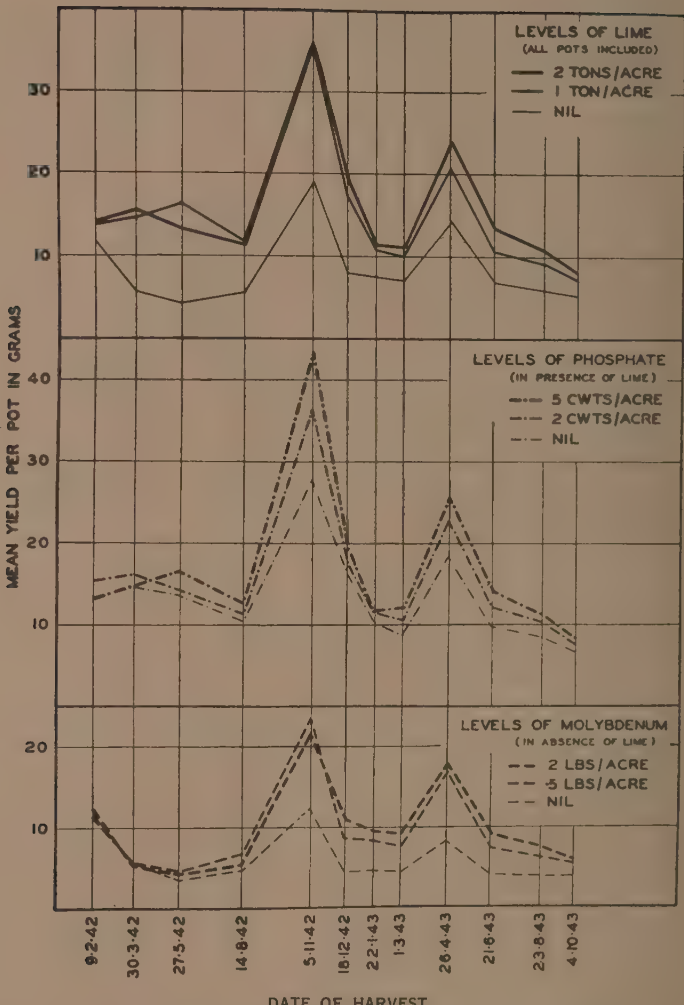

FIG. 1.—The effect of different levels of lime, phosphate, and molybdenum on the yield of lucerne grown in Duntroon soil.

Limed pots gave higher yields than unlimed pots at all harvests. The yield of the limed pots at the first harvest was slightly greater than that of the unlimed pots. At the second and third harvests, their yield was about three times as great as for the unlimed pots; and at subsequent harvests one and a half to twice as great. There was no difference between the yields from the two levels of lime for the first seven harvests, but subsequently the higher level (2 tons per acre) gave a consistently greater yield than the lower level.

Molybdenum had no effect on yield where lime was also added. On the unlimed pots, no significant increase in yield was obtained

until four harvests had been made, but at the fifth harvest molybdenum increased the yield by approximately 100 per cent. This increase was maintained at subsequent harvests. The higher level of molybdenum gave slightly higher yields than the lower level at the later harvests.

Phosphate had little effect on yield in the absence of added lime. On the limed pots there was a significant increase in yield due to phosphate at the third harvest and at all subsequent harvests. On the total yields for all harvests 2 cwt. of superphosphate per acre increased the yield of limed pots by 17 per cent., while 5 cwt. per acre gave a 29 per cent. increase.

TABLE 2.—TOTAL YIELD FROM EACH TREATMENT (12 HARVESTS) IN GRAMS PER 4 POTS.

Molybdenum lb./acre.	Lime—Nil.				Lime—1 ton/acre.				Lime—2 tons/acre.			
	Monocalcium Phosphate per Acre.			Total.	Monocalcium Phosphate per Acre.			Total.	Monocalcium Phosphate per Acre.			Total.
	Nil.	2 cwt.	5 cwt.		Nil.	2 cwt.	5 cwt.		Nil.	2 cwt.	5 cwt.	
Nil ..	270	265	311	846	625	697	837	2,159	646	747	820	2,213
½ ..	428	507	418	1,353	613	745	809	2,167	682	793	834	2,309
2 ..	443	487	504	1,434	597	700	792	2,089	656	781	824	2,261
Total ..	1,141	1,259	1,233	3,633	1,835	2,142	2,438	6,415	1,984	2,321	2,478	6,783

ANALYSIS OF VARIANCE OF TOTAL YIELDS IN GRAMS.

Source.	D.F.	Sum of Square.	Mean Square.	F.	Significance.
					%
Blocks	3	1,217	406	2·2	..
Treatments	26	210,638	8,102	43·3	0·1
Lime (Ca)	2	164,702	82,351	440·6	0·1
Phosphate (P)	2	20,053	10,027	53·6	0·1
Molybdenum (Mo)	2	6,454	3,227	17·3	0·1
Ca × P	4	6,251	1,563	8·4	0·1
Ca × Mo	4	11,187	2,797	15·0	0·1
Mo × P	4	1,348	337	1·8	..
Ca × P × Mo	8	643	80	0·4	..
Error	78	14,579	187	..	..

Minimum difference between total of 12 pots for significance at—

5 per cent is 133 g.

1 per cent. is 176 g.

0·1 per cent. is 229 g.

The total yields for each treatment are set out in Table 2. The analysis of variance based on these treatment totals shows that lime, phosphate, and molybdenum each had a highly significant effect on yield and that the effect of phosphate and molybdenum was dependent upon the presence or absênce of lime.

(iii) *Effect of Fertilizers on Chemical Composition.*

The material obtained from individual treatments at each harvest was analysed and the percentage protein nitrogen, calcium, and phosphorus was obtained. Only the values which indicate differences due to treatment are presented in the tables.

Table 3 (A) and Fig. 2 (A) show the effect of the different levels of lime and of molybdenum on percentage protein nitrogen. The mean percentage of nitrogen has varied from harvest to harvest, depending upon the stage at which the plants were cut. The effect of lime has been to increase the percentage protein nitrogen at all harvests after the first, this increase being greatest when the plants were harvested at maturity. Two tons of lime per acre gave no increase over one ton of lime per acre.

Molybdenum in the absence of lime also increased percentage protein nitrogen after the second harvest, but had no effect when lime was also present.

TABLE.—3.—CHEMICAL COMPOSITION.

Date of Harvest.	(A) Percentage Protein Nitrogen.					(B) Percentage Calcium.				
	Lime—Nil. Molybdenum/acre.			Lime.		Lime—Nil. Molybdenum/acre.			Lime.	
	Nil.	½ lb.	2 lb.	1 ton.	2 tons.	Nil.	½ lb.	2 lb.	1 ton.	2 tons.
9.2.42 ..	2·25	2·03	1·99	2·15	2·08	1·19	1·26	1·16	1·43	1·37
30.3.42 ..	1·64	1·82	1·50	2·78	2·66	1·30	1·35	1·41	1·69	1·70
27.5.42 ..	3·61	4·04	3·78	3·70	3·98	1·46	1·34	1·32	2·07	1·91
14.8.42 ..	3·51	3·93	4·04	3·69	3·91	1·62	1·55	1·55	2·03	2·15
6.11.42 ..	2·20	2·20	2·25	2·56	2·48	1·58	1·12	1·04	1·63	1·72
18.12.42 ..	2·47	2·81	2·98	2·84	2·81	1·24	1·07	1·14	1·31	1·49
22.1.43 ..	2·56	2·93	2·97	3·27	3·01	1·15	0·88	0·70	1·03	1·14
1.3.43 ..	2·57	2·94	3·28	3·19	2·94	1·04	0·94	0·91	1·37	1·64
26.4.43 ..	1·65	1·89	1·95	2·48	2·37	1·15	1·00	1·14	1·83	1·88
21.6.43 ..	3·27	3·34	3·56	3·41	3·49	1·22	1·34	1·39	1·80	1·98
23.8.43 ..	3·43	3·48	3·66	3·73	3·70	1·13	1·23	1·19	1·57	1·78
4.10.43 ..	3·10	3·59	3·46	3·45	3·53	1·18	1·24	1·24	1·61	1·91

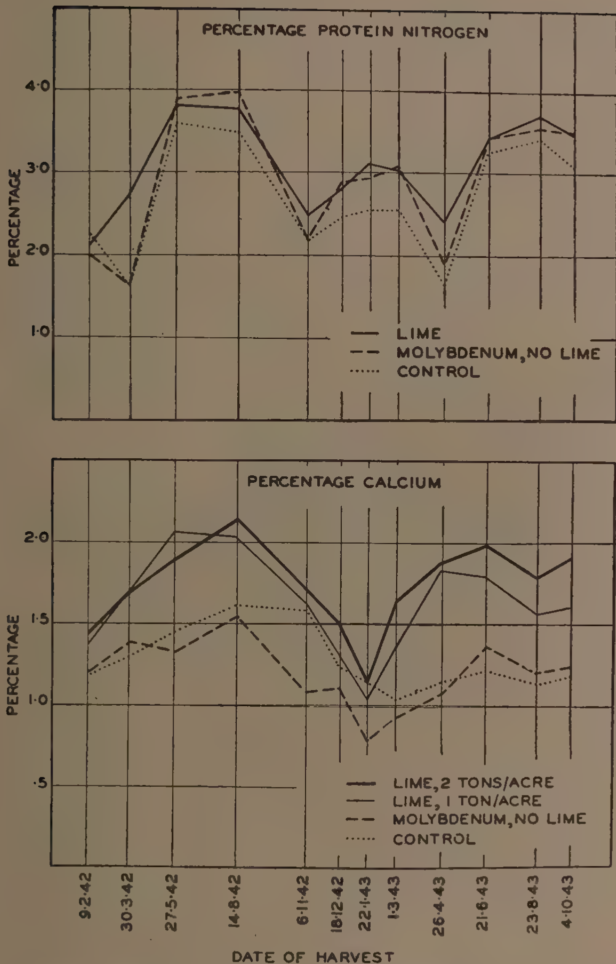

FIG. 2.—Effect of lime and molybdenum on (A) percentage protein nitrogen, and (B) percentage calcium content of lucerne.

Table 3 (B) and Fig. 2 (B) show the effect of these treatments on percentage calcium. Plants receiving lime were considerably higher in calcium at all harvests, and after the third harvest, the higher level of lime gave a consistently higher percentage of calcium than the lower level of lime.

Molybdenum had no effect on percentage calcium except at the fifth, sixth, seventh, and eighth harvests when plants receiving molybdenum and no lime were lower in percentage calcium than the controls (neither lime nor molybdenum). This is thought to be due to the limited amount of calcium available, and the higher yield of plants receiving molybdenum, together with rapid growth during that period.

Phosphate had no effect on percentage protein nitrogen or calcium.

Percentage phosphorus was increased by each addition of phosphate, and decreased by the addition of lime. The mean values for percentage phosphorus for the three levels of phosphate in the absence of lime were 0·27, 0·28, 0·33 respectively, and in the presence of lime 0·21, 0·23, 0·27.

4. Discussion.

The main effect on yield and health was obtained by the application of one ton of lime per acre. The application of lime in itself was sufficient to produce healthy and vigorous plants. It is not possible to state, on the basis of this experiment, whether this effect is due to the direct effect of the calcium, to the decrease in acidity resulting in better nodulation, hence more efficient nitrogen supply, or to the increased availability of other nutrients.

The Duntroon soil used in this experiment had a pH value of 5·2. The addition of one ton of lime per acre raised the pH to 5·8, and two tons of lime per acre to pH 6·5. Apparently the lime status and pH of the soil receiving one ton of lime per acre was satisfactory from the point of view of nodulation and supply of nutrients. No response was obtained from the extra ton of lime until a large amount of nutrients had been removed from the soil.

Lime may have acted indirectly by making other nutrients more available. The fact that molybdenum increased the yield after several harvests had been removed indicates that the soil is low in available molybdenum, though not necessarily in total molybdenum. The addition of lime alone made available sufficient molybdenum for normal growth of the plant, and no increase in yield due to added molybdenum was obtained on limed pots, even after twelve harvests had been made.

Mr. A. C. Oertel, of the Council's Division of Soils, analysed some of the harvested material spectrographically. The results indicate that the addition of lime increased the molybdenum content of the lucerne more than the addition of molybdenum itself, and that two tons of lime per acre increased the molybdenum content more than one ton of lime per acre. Lime also increased considerably the uptake of added molybdenum.

Response to phosphate is apparently dependent on an adequate lime status of the soil. Field experiments with both lucerne and subterranean clover have shown that, on areas where lime has little effect, the response to superphosphate is marked, and the plants are generally healthy. On areas where lucerne shows signs of yellowing, no response is obtained with superphosphate unless the lime requirement is first satisfied.

Responses to lime and molybdenum have been obtained by Anderson (1942), working on South Australian ironstone soils, and by Stephens and Oertel (1943) and Fricke (1944) with the Tasmanian Cressy shaley clay loam. However, in contrast to the present experiment, both Anderson and Fricke found that molybdenum alone had a much

greater effect than lime. Fricke concluded as a result of field experiments that the main effect of lime was to make available the molybdenum in the soil. The addition of lime and molybdenum together gave no increase over molybdenum alone.

Application of 2 lb. of molybdenum per acre has not increased the yield of stands of lucerne at Duntroon and Dickson, nor has it alleviated the deficiency symptoms. Moreover, in the pot experiment, the increase in yield due to molybdenum was only obtained after a succession of harvests, and even then was much less than the increase due to lime. In the plants receiving 2 lb. of molybdenum per acre, the percentage of molybdenum was much higher than that thought to be necessary for normal growth. It would appear that the effect of lime has not been merely to make additional molybdenum available from the soil, but that some other factor, or factors, including nitrogen, supplied or made available by lime, were limiting.

In another pot experiment, run at the same time, it was found that with lucerne growing in Duntroon soil, the number and size of nodules formed did not differ greatly in limed or unlimed pots, although the yield and percentage protein nitrogen were higher in plants from limed pots. If the effect of lime was to increase the nitrogen supply, then this must have been achieved by the operation of more efficient strains of bacteria, or by the bacteria working more efficiently in a favourable environment.

5. Acknowledgments.

The authors wish to acknowledge the fact that Mr. A. C. Oertel, M.Sc., of the Division of Soils, made spectrographic analyses of the molybdenum content of submitted samples.

6. References.

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Dormancy and Hardseededness in *T. subterraneum.*

4. Variation Between Varieties.

*By K. Loftus Hills, B.Agr.Sc.**

Summary.

From 5 to 21 varieties of subterranean clover were grown under similar conditions at Moss Vale in 1940 and 1941, at Canberra in 1941 and 1942, and at Melbourne in 1941.

The percentage of hard seed and the degree of delayed germination were determined for each lot. It was concluded that varieties differ in their tendency to produce dormant seed, but that hardseededness is not a varietal character or, if it is, the differences are of a low order and are usually masked by environmental conditions.

1. Introduction.

Although it is well recognized that dormant seed occurs in some species, but not in others, little attention has been paid to variation of the character within a species. Larson *et. al.* (1936) studied the relative dormancy of many varieties of wheat, oats, barley, and rye, and concluded that the length of the rest period required varied greatly with the variety. Burton (1940) investigated the germination behaviour of several lines of white clover. He showed that there were significant differences in response to cold storage, scarification, and temperature increase. No published information is available concerning differences in delayed germination between varietes of subterranean clover.

Aitken (1939) has drawn attention to the large variation in the proportion of hard seed in different varieties of subterranean clover grown under Victorian conditions. She observed that later strains appeared to contain more soft seed, and suggested that the relationship between the amount of hard seed and the relative maturity of varieties should be further investigated to determine if differences in hardseededness occurred apart from the secondary effects due to time of flowering.

The investigations described below were designed to determine whether varietal differences in dormancy and hardseededness occur in subterranean clover.

2. Material and Methods.

(i) *Delayed Germination.*

(*a*) *1940.*—Burr was harvested from nine varieties of subterranean clover grown as spaced plants at Moss Vale, N.S.W. The burr was threshed on a rubber hand thresher and the seed stored in packets in a laboratory drawer. Samples of 200 seeds were removed from each in April for testing of germination as previously described by the writer (Loftus Hills, 1942).

* An officer of the Division of Plant Industry.

(*b*) *1941.*—Twenty-one varieties were grown at Moss Vale, 16 at Canberra, and 5 at Melbourne. In some cases there were several lines of each variety, so that the total number of varietal lines at Moss Vale was 46, at Canberra 28, and Melbourne 7. They were grown under similar conditions at the three places, being arranged in three randomized blocks, each plot consisting of a number of spaced plants. The seed sown in each place was identical and was distributed from Moss Vale. Accurate flowering records were kept at Moss Vale where a plot was noted as flowered when half or more of the plants had at least one open flower. Every effort was made to harvest all the material at a similar stage of ripeness. It was then sent to Moss Vale where it was threshed and samples of 200 seeds counted out from each plot lot. The samples were then percussed and germinated in a laboratory cupboard in which a recording thermograph was placed. It had been intended to germinate the material in a germinator set at 20°C., but it was not large enough to take the 240 petri dishes. Otherwise the germination procedure was similar to that used previously by the writer (Loftus Hills, 1942). Certain varieties from each centre were tested a few months later at a constant temperature of 20°C.

(*c*) *1942.*—Nine of the varieties grown in 1941 were selected for a further trial at Canberra. On this occasion five replications of each variety were sown. The seed was sown directly into drills in the field, each plot being 15 feet long, and the plots 3 feet apart. Harvest and germination procedure was similar to that used in the previous year except that all germination tests were carried out at 20°C.

(*d*) *Presentation of Results.*—The number of days from the beginning of the germination tests to the stage where 60 per cent. of the swollen seed had germinated was read from curves for the 1940 data, and calculated by interpolation for the 1941 and 1942 series. The latter groups were analysed statistically.

(ii) *Hardseededness.*

(*a*) *1940.*—Samples of 200 seeds were counted out in April, 1941, from the seed lots described previously and germinated without further treatment. The hard seed was counted at the tenth day.

(*b*) *1941.*—Investigation had shown that threshing upon a rubber hand thresher sometimes affected the proportion of hard seed, probably by abrasion of the seed coat. It was therefore decided to remove the seed from the burr by the fingers. This was very laborious, and it was not possible to do all three replicates of each variety. As a compromise a composite sample of 200 seeds was built up from the three replicates of each variety. The seed was then germinated at 20°C., and counts of the hard seed made on the eleventh day.

(*c*) *1942.*—Seed threshed on the rubber hand thresher was used and 200 seed samples were tested from three of the five replicates of each variety. Counts of hard seed were made on the tenth day.

3. Results and Discussion.

(i) *Delayed Germination.*

The number of days required for 60 per cent. of the swollen seed to germinate, for all the varieties tested, is set out in Table 1. The daily minimum and maximum temperatures recorded in the cupboard

TABLE 1.—THE DEGREE OF DELAYED GERMINATION OF SEED OF VARIETIES OF SUBTERRANEAN CLOVER.

Variety.	Canberra.			Melbourne.		Moss Vale.			Moss Vale.
	1941.		1942.	1941.		1940.	1941.		1941.
	At 20° C. (Mar., 1942.)*	At Fluctuating Temperature (Jan., 1942.)*	At 20° C. (Feb., 1943.)*	At 20° C. (Mar., 1942.)*	At Fluctuating Temperature (Jan., 1942.)*	At 20° C. (Apr., 1941.)*	At 20° C. (Mar., 1942.)*	At Fluctuating Temperature (Jan. 1942.)*	Relative Flowering Time.
Mulwala	7·5	6·1	16·0	..	8·6	..	20·9	8·5 (2)†	+ 1
Bacchus Marsh ..	5·3	5·5	8·2	9·5	7·3	9·8	..	5·0 (2)	+ 20
Mt. Barker	11·6	6·2 (5)	14·6	18·6	8·5 (3)	20·9	..	6·5 (9)	+ 20
Macarthur	3·6	3·3	9·7	8·7	7·5	16·5	..	4·8	+ 34
Tallarook	2·0	4·9 (4)	6·8	5·4	7·0 (2)	8·3	..	5·1 (5)	+ 38
Burnerang	22·4	11·8	37·7	..	..	23·5	> 25·0	12·9 (2)	+ 37
Wenigup	1·9	2·0	10·4	..	..	10·2	1·9	2·0 (2)	+ 34
Wangaratta	..	8·5	26·4	..	..	..	..	8·3	+ 28
Dwalganup	2·7	6·3	9·7	..	..	..	..	4·9 (2)	0
Nangeela	..	5·9	..	..	..	14·6	..	7·6 (3)	+ 27
Kyneton	..	3·4	..	..	..	14·0	..	6·1 (2)	+ 43
Bass..	..	6·0	..	..	..	21·5	..	7·4	+ 46
Seaton Park ..	..	6·1	..	..	..	..	..	7·9	+ 2
Wallendbeen	..	4·9	..	..	..	..	..	6·8	+ 25
Gin Gin..	..	6·5	..	..	..	..	..	7·0	+ 20
Merino	..	6·1	..	..	..	..	..	6·5	+ 32
Orford	..	..	..	..	..	..	..	6·8	+ 26
Reigert's white seeded	..	..	..	..	..	..	..	7·2	+ 5
Romsey	..	..	..	..	..	..	..	4·9	+ 39
Second Northam ..	..	..	..	..	..	..	..	8·7	0
Yabba North.. ..	..	..	..	..	..	..	..	3·8	+ 5
Variance of log of mean of one line ..	..	·00154	.00177	..	.00154	..	..	.00154	..

* Tested during the month shown in brackets.
† The figures in brackets refer to the number of lines of the variety which have been averaged.

in which the varieties in columns 3, 6, and 9 of the table were germinated, are shown in Table 2. It will be observed that on the second day of those tests the minimum temperature was as low as 14°C. This appears to have resulted in a speeding up in germination of all varieties, for when several of them were tested at 20°C. two months later, presumably after considerable maturation would have taken place, germination was actually slower. However, we are concerned with relative rather than absolute values, and, although most of the varieties germinated fairly rapidly under these conditions of low and fluctuating temperatures, differences in germination still persisted. These differences are presumably indicative of the tendency of the varieties to produce dormant seed.

Table 2.—The Temperature of the Germination Cupboard at Moss Vale During the 1941 Tests.

Days from Commencement of Germination Test.	Maximum Deg. C.	Minimum Deg. C.
0	18	16
1	22	14
2	27	19
3	30	20
4	28	21
5	22	16
6	16	15
7	18	14
8	19	14
9	18	14
10	20	12

It is evident from an inspection of Table 1 that there are considerable differences in delayed germination between the several varieties within each group. The data have been analysed for four of the eight groups. A log x transformation was necessary to equalize the variances of the different varieties. The mean values given in the table are geometric means. The variances to be associated with the logarithms of the means of one line are given and the corresponding variances for n lines are, of course, the given values divided by n. The variety means differ significantly in each of the four groups. Although the other four groups were not analysed, it is evident that the differences in them are of the same general nature.

It is possible that differences observed between varieties under a particular set of environmental conditions may be due to a local interaction of variety with environment, which might not be duplicated elsewhere or in other seasons. The behaviour of the varieties under the five different sets of conditions has been compared by calculating the correlation coefficients for the ten possible comparisons. The values of r are set out in Table 3. In eight cases the values of r are significantly positive ($P = 0 \cdot 05$ or less), but although the other two values are also positive, they are not significantly so. However, none of the values falls below $+ 0 \cdot 445$, and, considering the data as a whole, it is evident that the varieties tend to behave in a similar manner in the several seasons and places.

In Table 4 the varieties in each group have been listed in order of increasing delay in germination. Although some modifications of order and degree do occur from group to group, it is possible to obtain a general idea of the tendency of the varieties to produce dormant seed. Thus seed of the varieties Wenigup, Tallarook, Macarthur, Bacchus Marsh, and Yabba North shows little delay in germination, whereas that of Burnerang, Wangaratta, and Second Northam is very resistant to germination. The commercial varieties appear to rank in the following order of decreasing dormancy—Mulwala, Mt. Barker, Dwalganup, Bacchus Marsh, and Tallarook.

TABLE 3.—THE CORRELATION COEFFICIENTS OF THE DELAYED GERMINATION OF SEED OF VARIETIES OF SUBTERRANEAN CLOVER GROWN UNDER PAIRS OF DIFFERENT CONDITIONS.

——	Moss Vale 1941.	Canberra 1941.	Canberra 1942.	Melbourne 1941.
Moss Vale 1940 ..	+ 0·738*	+ 0·651*	+ 0·802*	+ 0·926*
Moss Vale 1941 ..	..	+ 0·848*	+ 0·445†	+ 0·887*
Canberra 1941 ..	..	..	+ 0·875*	+ 0·605†
Canberra 1942 ..	..	..	..	+ 0·997*

* P = ·05 or less.
† Not significant.

The data presented so far have established that varietal differences in the tendency to produce dormant seed exist, and that these differences are fairly constant in different seasons and places. They may be a direct expression of an inherited character, or may be a secondary effect of other varietal characters such as differences in time of flowering. When both early and late varieties are grown together, seed of the later ones generally matures under hotter and drier conditions than does that of the earlier. Such a difference in conditions within an environment might account for varietal differences in behaviour of the seed, and being common to practically all places in southern Australia, might duplicate such differences elsewhere. Data concerning the flowering behaviour of the varieties grown at Moss Vale in 1941 are presented in column 10 of Table 1. It may be assumed that the order of flowering of the varieties in other places and seasons is approximately the same. There appears to be little, if any, relationship between the time of flowering and the degree of delayed germination of the varieties. This is confirmed by the fact that the value of the correlation coefficient between the two variables at Moss Vale in 1941 was not significant ($r = -\cdot007$). It may be concluded that differences in time of flowering are not responsible for the variation in delayed germination between varieties.

(ii) *Hardseededness.*

The proportion of hard seed produced by the varieties grown at Moss Vale in 1940 and 1941, at Canberra in 1941 and 1942, and at Melbourne in 1941 is shown in Table 5. Tests carried out with the 1940 seed showed that most of the varieties had reached a maximum hard seed content three months after harvest.

TABLE 4.—VARIETIES OF SUBTERRANEAN CLOVER ARRANGED IN ORDER OF INCREASING DORMANCY.

Canberra.			
1940 at 20°C.	1941 at 20°C.	1941 at Fluctuating Temperature.	1942 at 20°C.
Tallarook	Wenigup	Wenigup	Tallarook
Bacchus Marsh	Tallarook	Macarthur	Bacchus Marsh
Wenigup	Dwalganup	Kyneton	Macarthur
Kyneton	Macarthur	Tallarook	Dwalganup
Nangeela	Bacchus Marsh	Wallendbeen	Wenigup
Macarthur	Mulwala	Bacchus Marsh	Mount Barker
Mount Barker	Mount Barker	Nangeela	Mulwala
Bass		Bass	Wangaratta
Burnerang		Merino	Burnerang
		Mulwala	
		Seaton Park	
		Dwalganup	
		Mount Barker	
		Gin Gin	
		Wangaratta	
		Burnerang	

Melbourne.		Moss Vale.	
1941 at 20°C.	1941 at Fluctuating Temperature.	1941 at 20°C.	1941 at Fluctuating Temperature.
Tallarook	Tallarook	Wenigup	Wenigup
Macarthur	Bacchus Marsh	Mulwala	Yabba North
Bacchus Marsh	Macarthur	Burnerang	Macarthur
Mount Barker	Mount Barker		Dwalganup
	Mulwala		Romsey
			Bacchus Marsh
			Tallarook
			Seaton Park
			Mount Barker
			Merino
			Orford
			Wallendbeen
			Gin Gin
			Reigerts White Seeded
			Bass
			Nangeela
			Kyneton
			Wangaratta
			Mulwala
			Second Northam
			Burnerang

Analysis of variance of the 1942 figures showed that there were significant differences between varieties ($F = 9 \cdot 40$, $P = < \cdot 001$). Although the remainder of the data were not suitable for statistical examination, comparison with the 1942 figures, and with similar data obtained by other workers, suggests that real differences in hard seed content also existed at Moss Vale in 1940, and at Moss Vale and Canberra in 1941. It would appear that under suitable conditions all the varieties examined are capable of producing a high proportion of hard seed, for it will be observed that half of them produced over 90 per cent., and all of them over 75 per cent. of hard seed, in at least one of the five sets of conditions. The conditions at Moss Vale in 1940 resulted in the lowest proportion of hard seed, although the variation between varieties was greater than in the other seasons and places. It may be that varieties differ more under such conditions than under those in which a maximum proportion of hard seed is produced.

TABLE 5.—THE HARD SEED CONTENT OF VARIETIES OF SUBTERRANEAN CLOVER.

Variety.	Percentage of Hard Seed.					Relative Flowering Time.
	Canberra.		Melbourne.	Moss Vale.		Moss Vale 1941.
	1941 (Apr., 1942).*	1942 (Feb., 1943).	1941 (Apr., 1942).	1940 (Apr., 1941).	1941 (Apr., 1942).	
Mulwala ..	..	99·0	92·5	..	90·5	+ 1
Bacchus Marsh	82·5	79·8	95·0	67·0	87·8	+ 20
Mt. Barker ..	81·2 (6)†	91·5	95·3 (3)	68·3 (5)	75·5 (10)	+ 20
Macarthur ..	88·5	89·6	96·0	80·0	88·5	+ 34
Tallarook ..	85·2 (4)	90·7	98·5	69·7 (3)	88·0 (6)	+ 38
Burnerang ..	96·5	99·2	..	69·5	96·8	+ 37
Wenigup ..	89·5	89·6	..	36·8 (2)	91·5 (2)	+ 34
Wangaratta ..	90·0	95·3	..	..	89·5	+ 28
Dwalganup ..	95·0	..	95·5	..	90·0	0
Nangeela ..	84·0	..	..	82·0	87·3	+ 27
Kyneton ..	68·0	..	..	..	80·5	+ 43
Bass	70·5	..	..	51·5	85·5	+ 46
Seaton Park ..	86·5	..	..	..	77·5	+ 2
Wallendbeen ..	77·0	..	..	..	77·5	+ 25
Gin Gin ..	75·0	..	..	..	75·0	+ 20
Merino ..	90·5	..	..	..	84·5	+ 32
Orford ..	81·5	..	..	..	87·5	+ 26
Reigert's white seeded ..	86·5	..	..	..	82·0	+ 5
Romsey ..	90·5	..	..	..	92·0	+ 39
Second Northam	..	..	..	..	87·0	0
Yabba North ..	..	..	..	..	89·5	+ 5
S.E.	..	2·06	..	..	..	..

* Tested during the month shown in brackets.

† The numbers in brackets refer to the number of lines of the variety which have been averaged.

In order to determine whether the varietal variation in hard seed content was consistent under the five different sets of conditions, the value of the correlation coefficient (r) has been calculated for the ten possible comparisons. The values are set out in Table 6. In eight

of the ten there is no significant correlation. There is a fair probability (P = ·07) of the existence of a positive relationship between the hard seed content of varieties in the two successive seasons at Canberra, whilst there is a high probability (P = ·005) of a similar relationship existing between Moss Vale and Canberra n 1941.

In Table 7 are shown the values of r for the correlation between the relative flowering times of the varieties and their hard seed contents, in each of the five environments. In four cases there is no significant relation. However, there is evidence of a positive relationship (P = ·07) between the two characters at Melbourne in 1941, but as the range of hard seed contents was very small, the number of varieties so few, and as there is a probability of one in three that such a correlation would occur by chance once or more in five cases, the writer feels that the association should be regarded with reserve.

TABLE 6.—THE CORRELATION COEFFICIENTS OF THE PROPORTION OF HARD SEED PRODUCED BY VARIETIES OF SUBTERRANEAN CLOVER GROWN UNDER PAIRS OF DIFFERENT CONDITIONS.

——	Moss Vale, 1941.	Canberra, 1941.	Canberra, 1942.	Melbourne, 1941.
Moss Vale, 1940	− ·111*	+ ·180*	+ ·076*	+ ·086*
Moss Vale, 1941	..	+ ·635†	+ ·325*	− ·091*
Canberra, 1941	..	..	+ ·718‡	+ ·007*
Canberra, 1942	..	..	..	+ ·344*

* Not significant.
† P = ·005.
‡ P = ·07.

TABLE 7.—THE CORRELATION COEFFICIENTS OF THE PROPORTION OF HARD SEED PRODUCED BY VARIETIES OF SUBTERRANEAN CLOVER, AND THEIR RELATIVE FLOWERING TIMES.

Moss Vale, 1940.	Moss Vale, 1941.	Canberra, 1941.	Canberra, 1942.	Melbourne, 1941.
− ·332*	+ ·153*	− ·272*	− ·122*	+ ·770†

* Not significant.
† P = ·07.

The data presented are not conclusive, but they suggest that, if hardseededness is a varietal character, then the differences between varieties are of a low order and are subject to change in different environments. The relative maturity of varieties did not affect their hard seed content, and it is therefore suggested that climatic fluctuations

during the maturation period are responsible for the interaction of variety and environment. Possibly atmospheric conditions and/or soil moisture have a marked influence during some critical phase of the maturation process.

4. Conclusions.

(i) The tendency of subterranean clover to produce dormant seed is a varietal character.

(ii) The differences in delayed germination between the varieties are generally maintained in different seasons and places, although other factors may sometimes modify their direction and degree.

(iii) The commercial varieties rank in the following order of decreasing dormancy:—Mulwala, Mt. Barker, Dwalganup, Bacchus Marsh, and Tallarook.

Of the 21 varieties observed, Wenigup, Tallarook, Macarthur, Bacchus Marsh, and Yabba North showed the least tendency to produce dormant seed, and Burnerang, Wangaratta, and Second Northam, the greatest.

(iv) The varieties of subterranean clover did not differ consistently in the proportion of hard seed which they produced.

(v) The differences in hard seed content that occurred in specific seasons and places were generally not related to the relative flowering times of the varieties.

5. Acknowledgments.

The help given by Miss Yvonne Aitken of the University of Melbourne both in growing material and in discussion, particularly of the hard seed problem, is gratefully acknowledged. The writer also wishes to thank Mr. G. A. McIntyre for help in the statistical examination of the results.

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Dormancy and Hardseededness in *T. subterraneum.*

5. The Effect of the Condition of the Seed Coat upon Embryo Dormancy.

*By K. Loftus Hills, B.Agr.Sc.**

Summary.

Experiments were carried out to determine whether the speed of germination of dormant seed of subterranean clover, which was permeable to water, was effected by the condition of the seed coat. It was concluded that dormant seed will germinate rapidly in the absence of the coat, but that the speed of germination is the same whether the seed is softened by percussion or by scarification. No difference was demonstrated in the speed of germination of naturally soft seed, and of naturally hard seed which had been softened by artificial means.

1. Introduction.

It was pointed out in the first paper of this series that new season's seed of subterranean clover often fails to germinate either because the seed coat is impermeable to water, or because the embryo† is physiologically immature. It is not known whether these two conditions are related; although it seems reasonable to assume that the physiological condition of the embryo would not affect the permeability of the seed coat. However, it is possible that the condition of the seed coat would affect the degree of delayed germination shown by the embryo. Such an effect might be direct in that the degree to which the seed coat acts as a shield to the ingress of light, and/or moisture, and/or the atmosphere, etc., could influence the behaviour of the embryo. Alternatively there might be an indirect relationship in that maturation conditions which affect the development of the seed coat might also affect the development of the embryo. We should then expect that those seeds which are permeable to water owing to the incompletion of the permeable layer, would have a different degree of embryo dormancy, and hence of delayed germination, than would those seeds in which the suberization of the impermeable layer is complete.

The writer has carried out preliminary experiments in an endeavour to answer the above questions. It has proven difficult under experimental conditions to separate the effects of embryo dormancy and of hardseededness, and the data which are presented below must be considered as having only partially solved the problem.

2. The Effect of the Removal of the Seed Coat.

The seed coats were removed by dissection from 77 seeds which had been harvested a week or so before harvest maturity. There were 30 seeds of Tallarook and Mt. Barker, and 17 of Dwalganup. The seeds

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* An officer of the Division of Plant Industry.

† It is generally assumed that the embryo is the site of the physiological changes involved in after-harvest ripening, but it is possible that the adjacent tissues also play a part.

were germinated in the usual manner. The germination results together with those obtained by germinating the seed under similar conditions with the seed coats scarified, but not removed, are set out in Table 1. Seventy-six of the seeds from which the coats had been removed had germinated by the third day. At the same stage of the test less than 5 per cent. of the intact scarified seed had germinated. It will be observed that a proportion of the seedlings was classed as abnormal. They were stunted and somewhat similar to those obtained by Flemion (1938) from embryos of peach, *Rhodotypos*, etc., which had been completely removed from the seed.

It is evident that in subterranean clover the seed coat plays an important part in retarding the germination of dormant seed, even though it is permeable to water.

TABLE 1.—THE GERMINATION OF DORMANT SEED OF SUBTERRANEAN CLOVER FROM WHICH THE SEED COATS HAVE BEEN REMOVED.

Variety.	Seed Coats Removed.					Seed Coats Scarified but not Removed.	
	Number of Seeds.	Number of Seeds Germinated.				Germination of Swollen Seed.	
		After 3 Days.		After 21 Days.		After 3 Days.	After 21 Days.
		Normal Seedlings.	Abnormal Seedlings.	Normal Seedlings.	Abnormal Seedlings.	All Seedlings Normal.	
						%	%
Mount Barker ..	30	18	12	20	10	< 5	< 5
Tallarook ..	30	30	0	30	0	< 5	90
Dwalganup ..	17	3	13	5	11	< 5	28

3. The Effect of Different Methods of Artificially Softening the Seed.

Two types of seed-coat treatment were compared. The first was a vigorous scarification with medium-grained sandpaper, and the second percussion by mechanical shaker for five minutes, as described in the first paper of this series (Loftus Hills, 1942). The former treatment acts by removing patches of the surface impermeable layer, whilst the latter opens a small line in the impermeable layer, known as the strophiole (Aitken, 1939). The two treatments were combined to give a third additive treatment. Two independent experiments, the results of which are summarized in Table 2, were carried out. There were no significant differences in delayed germination between the three seed-coat treatments.

TABLE 2.—THE RELATIVE EFFECT OF PERCUSSION AND SCARIFICATION UPON THE DELAY IN GERMINATION OF SEED OF SUBTERRANEAN CLOVER.

Variety.	Number of Days Required for 60 per cent. of the Swollen Seed to Germinate.		
	Percussed.	Scarified.	Percussed. and Scarified.
First Series—			
Tallarook	4·0	3·8	3·4
Burnerang	13·5	14·4	17·6
Mount Barker	4·2	4·7	5·2
Second series—			
Tallarook	3·3	4·0	3·1
Mount Barker	6·8	6·1	9·3
Bacchus Marsh	3·8	5·3	4·1

For the second series, F = 0·3503, hence the treatment means are not significantly different.

4. The Degree of Embryo Dormancy of Naturally Soft and of Naturally Hard Seed.

The object of this series of tests was to discover if those seeds which had experienced maturation conditions resulting in incomplete suberization of the outer seed coat layer, differed in embryo dormancy from the naturally hard seed in the same sample, when the latter were softened by artificial means. The logical procedure would be to separate the sample into hard and soft groups and then to germinate them after percussion or scarification. This was impracticable, so a compromise was adopted whereby the germination behaviour of the untreated sample was compared with that of the whole sample after it had been percussed. The germination percentage of the swollen seed in the naturally hard group of the population was then obtained from the equation :—

$$G_h = \frac{100\ G_t - S\ G_s}{H}$$

where G_h = percentage germination of the hard seed group
G_s = percentage germination of the soft seed group
G_t = percentage germination of the whole sample
S = percentage of soft seed in the sample
H = percentage of hard seed in the sample.

The results of the tests, which were carried out in two successive seasons, are set out in Table 3. The fourteen separate lots of seed which were compared in 1941 behaved in a variable manner, and the mean values were not significantly different. In the 1942 results there is a slight probability that the hard seed group is less dormant than the soft. However, an unverified assumption, viz. that unshaken naturally soft seed behaves similarly to shaken naturally soft seed, is inherent in the method used. Nevertheless, the data indicate that it is improbable that the embryos of naturally soft seed are less dormant than are those of naturally hard seed.

5. Conclusions.

(*a*) The seed coat of subterranean clover, even when permeable to water, inhibits the germination of the physiologically immature embryo. In very dormant material a proportion of the resulting seedlings may appear abnormal.

(*b*) There was no difference in the delayed germination shown by percussed or by scarified seed.

(*c*) It is improbable that the embryos of naturally soft seed are less dormant than those of naturally hard seed.

TABLE 3.—THE DEGREE OF EMBRYO DORMANCY OF NATURALLY SOFT AND OF NATURALLY HARD SEED OF SUBTERRANEAN CLOVER.

(*a*) 1941—

Total Number of Seeds.	Hard Seed.	Soft Seed.	Germination of Swollen Seed.		
			In Complete Sample.	In Soft Seed Group.	In Hard* Seed Group.
	%	%	%	%	%
2,800	59·2	40·8	37·6	44·0	33·2

t = 0·32, hence the treatment means are not significantly different.
By calculation.

(*b*) 1942—

Variety.	Hard Seed.	Soft Seed.	Germination of Swollen Seed.		
			In Complete Sample.	In Soft Seed Group.	In Hard* Seed Group.
	%	%	%	%	%
Tallarook ..	90·2	9·8	72·2	28·2	77·0
Mount Barker ..	75·0	25·0	23·1	22·2	23·4
Bacchus Marsh	79·7	20·3	60·6	13·5	72·6

t = 2·04 : P = > 0·10.
* By calculation.

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Dormancy and Hardseededness in *T. subterraneum.*

6. The Application of the Results to the Problem of Re-establishment in the Field and to Seed Testing and Marketing.

*By K. Loftus Hills, B.Agr.Sc.**

Summary.

The bearing of the results presented in the first five papers of this series upon the problem of re-establishment of subterranean clover in the field, and upon seed testing and marketing practices, is discussed.

It is concluded that environmental factors are chiefly responsible for the time of re-establishment, and that it is not likely that an improvement programme designed to procure varieties with earlier re-establishment characteristics would be worth while. Certain suggestions are made concerning modifications in seed testing technique which would result in a greater emphasis being placed upon delayed germination and hardseededness.

1. Introduction.

The district of Moss Vale, New South Wales, has a mean annual rainfall of about 35 inches, which is fairly evenly distributed throughout the twelve months. The more fertile soils in the area will generally support perennial white clover, but the poorer soil types will not do so. The usual recommendation in such cases is that subterranean clover be sown in place of, or in conjunction with, the former species. However, subterranean clover is a winter annual having a considerable interval between seeding and re-establishment. In areas of little or no summer rainfall this is an advantage, because not only does the seed burr form a valuable summer fodder, but as the seed does not germinate after an occasional summer shower it does not re-establish prematurely.

The position in such environments as Moss Vale is somewhat different because the rest of the sward may continue to grow during the summer. The ground previously covered by subterranean clover may remain unproductive, and the summer rainfall may leach and otherwise accelerate the loss of the residual dry matter. Under such circumstances, it seems desirable that the species should re-establish as soon after formation of the seed as is consistent with the flowering of the plants at the normal time in the following spring.

Before attempting to select varieties of subterranean clover with different re-establishment characteristics, it appeared desirable to find out what factors controlled the re-establishment of the species, and whether a breeding programme was likely to be successful. The ultimate objective would be to select or breed varieties with specific re-establishment characteristics which were suited to particular rainfall distributions.

* An officer of the Division of Plant Industry.

The principal results of the investigations have been reported in the first five papers of this series. The present article discusses the bearing of them upon seed testing and marketing practices, upon re-establishment in the field, and upon the problem of altering the time of re-establishment by breeding or selection of new varieties.

2. Seed Testing and Marketing.

With the present methods of testing seed of subterranean clover, no cognizance is taken of the variation in delayed germination in different samples. Very dormant seed is germinated at low temperatures, and it is tacitly assumed that such seed will have fully matured by the time it is sown by the farmer. The evidence presented in the second paper of the series suggests that this might not always be so.

The speed of germination of most agricultural seeds is particularly important under Australian conditions, where a heavy germinating rain is often quickly followed by severe drying conditions. In such circumstances, seeds should be capable of germinating in a matter of hours rather than of days, if the roots are to reach the sub-surface soil moisture before the surface zone dries out. The speed of germination may then be of greater importance than the final percentage given by the usual germination test. If it were possible to differentiate between samples in regard to delayed germination it would serve as an incentive to the seed trade to avoid dormant lots, or at any rate, to hold over such seed until the following season. Indirectly, it would force the seed grower to take any steps, such as delaying the harvest of the seed crop, which might reduce the chance of obtaining dormant seed.

It is probable that an unnecessarily high proportion of hard seed is contained in many commercial seed lots of subterranean clover. It is held that a certain proportion of hard seed is desirable as an insurance against failure of the first germination, but it seems unlikely that a substantial proportion of such seed would soften during the few weeks concerned. A better practice would be to resow and thus ensure a good clover cover the following spring. Any residual hard seed is virtually useless, as by the time it has the opportunity of useful germination there will be ample seed available from the established plants. Hardseededness is simply overcome by mechanical means, and merchants should be encouraged to process seed containing more than, say, 20 per cent. of hard seed.

In view of these facts, it is suggested that the germination requirements for subterranean clover seed might be modified in the following manner. In addition to the present standards for weed seed impurities, etc., and for total germination after ten days at 20°C., there should be a maximum permissible content of hard seed, and a minimum speed of germination. The latter might best be expressed in terms of the time required for an empirical percentage of the swollen seed to germinate. It is tentatively suggested that the maximum time allowed for the germination of 60 per cent. of the swollen seed at 20°C. should be five days, and that samples should contain 30 per cent. or less of hard seed.

3. Field Re-establishment.

The principal factors influencing field re-establishment of subterranean clover are hardseededness, embryo dormancy, surface soil moisture, and surface soil temperature.

The proportion of hard seed may increase for a week or so after the stage of harvest ripeness, but within a few weeks it begins to decrease. In most cases, there should be sufficient permeable seed available a month after harvest ripeness to give an adequate ground cover, provided the other factors are favourable for germination. Thus, it is not considered that hardseededness is a bar to early summer re-establishment.

Embryo dormancy is probably the chief inhibiting factor, although its expression is conditioned, to a large extent, by the amount and duration of surface soil moisture, and by surface soil temperature. It seems that at the stage of harvest ripeness the seed of even the least dormant varieties requires either a low temperature or a long period of adequate moisture if it is to germinate. As after-harvest ripening proceeds, the germination requirements of the seed in regard to temperature and time become less exacting, and the surface soil temperatures decrease, until by the autumn the seed is usually able to germinate within 48 hours after a fall of rain. Although reference to the monthly mean minimum temperatures of such areas as Moss Vale might suggest that there is a fairly high expectation of encountering temperatures as low as 10°C. in early summer, it must be remembered that such temperatures are only reached for short periods, and that surface soil temperature may be several degrees above the grass reading. The chances of encountering adequate soil moisture, together with sufficiently low temperatures for the required length of time in early summer, are not great, but such conditions undoubtedly could and do occur from time to time. At Moss Vale in 1941, conditions were such that the seed was capable of rapid germination by the middle of February. A substantial fall of rain then took place and excellent re-establishment was evident within five days.

It has been established that the varieties differ in the tendency to produce dormant seed, but such differences are considered to be of secondary importance, as the time of re-establishment is primarily dependent on environmental conditions. However, it is possible that in special circumstances it may be desirable to use varieties with different dormancy characteristics. A hypothetical case, for example, is that of the use of the species under irrigation, where it might conceivably be necessary to water the sward in early summer and at the same time hold back the germination of the subterranean clover. In such a case, an extremely dormant variety might prove of value.

Generally speaking, re-establishment is controlled by the environment, both through the effect of atmospheric moisture and temperature on the rate of after-harvest ripening of the seed, and through the germination environment provided by rainfall and temperature. It may be possible to modify the effects of these dominant factors in special circumstances, by the use of either extremely dormant or non-dormant varieties. However, the writer does not think it probable that substantially earlier summer germination could be obtained in such areas as Moss Vale by the use of less dormant varieties.

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Progress of Work with Potato Stocks Free from Virus X (FX Potatoes).

*By J. G. Bald, M.Agr.Sc., Ph.D.**

Summary.

This is an interim report on the progress of what has become known as the FX potato plan. The FX Up-to-Date potatoes have been multiplied sufficiently to supply the basic stocks in a permanent scheme for multiplication and distribution of FX certified seed. Trials have been made of the yielding ability and characteristics of the different families. FX Up-to-Date gave substantial increases in yield over certified Up-to-Date. Large scale multiplication of FX Bismarck has begun, and Snowflakes free from virus X have been found. An outline is given of the FX plan.

1. Introduction.

In the first report on the isolation of potatoes of the variety Up-to-Date free from virus X†, a provisional programme for future work on this problem was outlined. Firstly, the virus-free potatoes already obtained were to be multiplied as clonal stocks and tested for their agronomic qualities. Secondly, a search was to be made for virus-free stocks of the other five most widely grown Australian varieties and, if any were found, they too were to be multiplied and tested. Some progress has been made in both these phases of the programme; in addition, a plan for the permanent maintenance and distribution to certified seed growers of potato seed stocks free from virus X has been prepared and is now coming into operation.

To distinguish these stocks from certified seed carrying virus X, their description will always include the prefix FX (free from X). Only seed stocks and varieties officially included in the scheme will be given this designation.

2. FX Up-to-Date Seed Stocks.

Of the thirteen clones originally isolated, one (No. 117) has been at least temporarily discarded because it has some "bolter" characteristics. Stocks of it are being maintained in case it proves to have valuable qualities.

It is becoming clear that there are definite agronomic differences amongst the other twelve clones. The most striking is shown by No. 265 which is of relatively late maturity, and under good growing conditions in southern districts it can be distinguished by its foliage characteristics. It has a slight rugosity of the leaf surface and a slightly lighter colour than other clones. We had almost decided to discard it because of a rather low yield and the typical foliage, when results from a trial in northern New South Wales showed that, there, it held promise of being the highest yielding of all the clones.

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* An officer of the Division of Plant Industry.

† Bald, J. G. (1941).—Obtaining virus-free potatoes. *J. Coun. Sci. Ind. Res. (Aust.)*, 14: 187-90.

In the summer of 1941-42, samples of the twelve FX clones were grown by Mr. C. E. W. Oldaker, Agronomist, and Mr. A. Cooper, Farm Overseer, at the Tewkesbury Potato Station in Tasmania. Observations were made on type and maturity. The twelve clones were divided by Mr. Oldaker and Mr. Cooper into seven maturity classes, the earliest and latest of which differed by two to three weeks. Since then, the seasons and the state of the crops have made it impossible to observe such fine differences, but when, last season, a replicated yield trial was conducted at Tewkesbury, it was found that the yields ran roughly parallel to the previous maturity classification. Earlier maturing clones were, on the whole, lower yielding.

From this and other evidence it is clear that agronomic differences amongst the clones of FX Up-to-Date do exist, but it will take a number of years to define the differences, and to find which clones are most suited to the various regions in which Up-to-Date potatoes are grown.

Since the first thirteen virus-free Up-to-Date tubers were isolated, other X-free stocks of the same variety have been found. In Tasmania, Dr. R. A. Scott, over a period of years, made single plant selections of Up-to-Date on the farm of Mr. R. G. Tole at Myrtlebank. The selections were made in the field on a basis of yield, tuber type, and freedom from disease. They were multiplied, some being maintained as clones, and those that appeared less promising were bulked. During 1943, sample tubers were send to the author for testing, and it was found that with the exception of the bulked lots, the selections were free from virus X. The bulked material contained a proportion of X-free tubers. By field selection under favourable conditions, the same result had been reached as by inoculation tests in the greenhouse.

In Victoria, during the inspection of a crop of Snowflake potatoes, an Up-to-Date plant, which had the appearance of complete health, was found as a rogue in the crop. It was discovered by inoculation tests to be virus-free. It thus seems possible, if further FX Up-to-Date clones are wanted, that they can be isolated from commercial stocks.

Before the FX plan was finally put into operation, two points had to be determined. These were whether the FX stocks were actually higher yielding than certified stocks, and whether they could be retained reasonably free from virus X when they were being grown under ordinary farming conditions. A series of yield trials during 1943-44 showed gains by the FX stocks over the best certified stocks of between 14 and 27 per cent., except in one instance, where there was evidence of an effect of the source and condition of the seed tubers on yield. In this trial the yields of three FX clones grown from seed tubers from another district were less than that of local X-infected certified seed. The increases over certified seed in total yield produced by locally-grown FX clones varied between 3 and 10 per cent., but the increases in the yield of tubers over 3 oz. were between 11 and 20 per cent.

All indications so far are that the FX stocks can be grown for several successive years on farms under commercial conditions without showing any signs of infection. Spot tests have failed to reveal the presence of virus X in such crops, and they have retained the colour, vigour, and uniformity that were characteristic of the clones when

they consisted of only a few plants. Parent stocks maintained at the Tewkesbury Potato Station and at Canberra have yielded well and retained their freedom from virus X.

The amount of FX Up-to-Date seed tubers available for planting next season will probably exceed 50 tons. This is far less than was hoped for. During 1943, the produce of a crop planted in the winter months at Gatton, in Queensland, comprising the larger portion of the stocks then available, was lost from transit rots. These were due to wartime delays on the rail journey south during exceptionally hot and humid weather. However, a plot of 4 acres planted in South Australia during the winter yielded about 2½ tons to the acre, and the tubers from this crop, grown, after dormancy treatment with acetylene, in late districts of Victoria yielded well.

Officers of the following departments have co-operated in the multiplication of the FX Up-to-Dates: the Tasmanian, New South Wales, South Australian, and Victorian Departments of Agriculture, and Lawes Agricultural College, Queensland.

3. FX Bismarck Seed Stocks.

Officers of the Tasmanian Department of Agriculture have for a number of years been making selections of the improved Silverskin Bismarck and, by this means, have freed the certified stocks from serious infection with virus A. Virus A in combination with virus X had previously been causing such losses in yield that the variety had been threatened with commercial extinction. Examination of the improved mother seed stocks at the Tewkesbury Potato Station showed them to be infected with virus X. In a plot of single plant selections from these stocks, the presence of several plants free from all symptoms was noticed. Two tubers were chosen from one of these plants, taken to Canberra, and submitted to the usual tests for virus infection. They were proved to be virus-free. They were then multiplied by sprouting the tubers in the greenhouse, removing the sprouts after they had formed roots and the first leaves, and planting the shoots in pots. The potted plants were later set out in the field. In this way the initial multiplication was greatly hastened. Subsequently, this Bismarck clone was multiplied under field conditions in the ordinary way. By next season, there will be sufficient seed available to plant about 2 acres.

No field trials have yet been conducted with the FX Bismarck, because the material is too valuable to risk, during the early stages of multiplication, by growing it beside X-infected stocks. Yield trials are planned for the coming season. The general appearance of health and vigour, and the yield and quality of the tubers are so outstanding that it has been decided, without further evidence of their superiority, to incorporate the variety Bismarck in the FX scheme.

4. Stocks of Other Varieties Free from Virus X.

During January and February, 1943, crops of Snowflake in Victoria were examined for evidence that they might contain virus-free plants. In this variety under cool conditions virus X causes a visible mottle. The better crops appeared to be universally infected with virus X.

One small patch of several acres was found which had been grown from tubers bought on the open market. It contained a high percentage of diseased and unthrifty plants and rogues, but it also contained a few Snowflake plants of outstanding health and vigour. Ten that appeared free from the slight mottle induced by virus X were marked, and after they were harvested, the tubers of five were found to be virus-free. This material has been taken over by the Victorian Department of Agriculture for multiplication.

Two modern American varieties that hold promise of usefulness under Australian conditions are Katahdin and Sebago. Samples of these were received by the Council from America in a virus-free condition, and have been maintained virus-free. Other stocks have entered the country, and although Katahdin shows little sign of infection with virus X, crops of Sebago in Victoria have been found to contain infected plants. There is a probability that infection will spread in both varieties unless care is taken to eliminate virus X from parent stocks. Single plant selections have been made in Victoria and Tasmania by departmental officers and have been tested for virus X. Any infected plants have been eliminated and the rest are being multiplied in isolation.

Stocks of other varieties not of commercial importance, or not yet tried under Australian conditions, have been isolated in a virus-free condition. New introductions are being tested for their virus content as they are received.

Trials involving thousands of plants and tubers, and inspection of many crops in the field, have so far failed to yield virus-free stocks of the varieties Carman (possibly synonymous with Green Mountain) and Brownell (Adirondack). Trials of the variety Delaware (probably Earliest-of-All) on a similar scale have also failed.

Summarizing, three of the six varieties that make up by far the greater portion of the Australian potato crop, and the two most promising varieties recently introduced, have been obtained in a virus-free condition. Two varieties, Up-to-Date and Bismarck, have been included in the FX scheme, and others will be considered for inclusion when sufficient stocks known to be free from virus X are available for tests of their agronomic qualities.

5. The FX Plan.

Only the first stages of the plan for maintaining and multiplying the FX potato stocks have yet been put in operation. Many details are yet untried. The plan will be described only in sufficient outline to make the general principles of its operation clear.

Its central feature is the continual selection and testing of a nuclear stock of a variety, to keep it free, as far as possible, from all virus infection; and the continual renewal of certified seed by the multiplication of the nuclear stock. The first two stages in the multiplication of an FX stock are performed at a potato station by technical officers, and the third either at potato stations or on selected farms under the supervision of technical officers. Thereafter, the stock is taken over by certification organizations and multiplied by growers of certified seed.

The crops in the first two years are called the foundation and isolation blocks. The foundation block is planted from, say, 300 plants selected during the previous season from the isolation block of that season. When the attributes of the different clones of a variety are known, the selected plants will be chosen in the field from amongst the clones considered most desirable for the regions in which the certified seed and table potatoes will ultimately be grown. Each selected plant is tested for the presence of virus X; at present this is done by inoculation to *Datura stramonium*. Provided the selected plants have grown well during the season, and neither tops nor tubers have shown any sign of disease, their yields are stored separately for planting as single hill units in the foundation block of the following season. At planting, tubers are cut into the maximum number of seed pieces, and set in the field at wide spacing to produce the maximum yield. During the growing season, the block is frequently inspected, and any unthrifty plants are rogued.

The question of isolation has received some attention, because Australian experience agrees with published results of trials in Maine*, that small plots planted considerable distances away from other potatoes often serve as a focus of attraction for winged aphids carrying such diseases as leaf roll. The central portions of large blocks of potatoes are less liable to infection from such sources, because flying aphids settle mostly on the edges of the blocks. Also, relative to the number of plants, fewer winged aphids are likely to colonize a large isolated block of potatoes than a small one. Advantage is being taken of these facts by planting the foundation blocks in the centre of the isolation blocks. Further, of the two varieties so far taken into the FX plan, Bismarck is susceptible to virus A and Up-to-Date is field immune; Up-to-Date is very susceptible to leaf roll, and Bismarck has a useful degree of resistance. Therefore, the foundation block of Bismarck is being planted in the centre of the Up-to-Date isolation block, and vice versa. Thus, the foundation blocks are buffered against chance visits from a distance by infective winged aphids, and are protected against aphis-borne infection from the isolation blocks by the differences in susceptibility of the two varieties.

The entire yield of the foundation block is held until the next season to serve as seed for the isolation block. Tubers from the single hill units in the foundation block are bulked, but clones are kept separate. Isolation from other crops containing virus X and aphis-borne virus diseases is being strictly observed.

The production target for the isolation block is a yield of 15 to 20 tons. After digging, the whole yield of the isolation block, except of the plants chosen to provide the seed for the foundation block during the following season, is to be divided between the States in which the variety under consideration is grown. The seed tubers will be used to plant multiplication blocks, which will be the basis for the renewal of seed stocks by the growers of FX certified seed potatoes. These growers will be required to renew their seed regularly from crops derived from the multiplication blocks, instead of carrying on their own seed for an unlimited period of years.

* Folsom, D. (1942).—Potato virus disease studies with tuber-line seed plots and insects in Maine, 1927 to 1938. Maine Agric. Exp. Sta., Bull 410.

The finance and general administration of the FX plan have been taken over by the Federal Department of Commerce and Agriculture. The cost of growing the foundation and isolation blocks is being borne by the Commonwealth Government. The tubers from the isolation blocks are given, free of cost, to State Departments of Agriculture for distribution to farmers or stations where the multiplication blocks are grown.

The detailed administration of the scheme is controlled by the State Departments of Agriculture through their potato and certification branches. The foundation and isolation blocks of Up-to-Date and Bismarck, the only two varieties so far incorporated in the FX scheme, are being grown at the Tewkesbury Potato Station in Tasmania, where mother stocks for certified Brownell and Bismarck potatoes have been held for a number of years. The officers in charge of the Tasmanian certification scheme control the field work of the foundation and isolation blocks. The testing of selected plants for freedom from virus X is at present being shared by the Tasmanian Government Plant Pathologist and officers of the Council in Canberra. The scheme is a co-operative enterprise involving, in the first stages, officers of State and Federal Departments, and in the later stages farmers and certification organizations. It was designed to make use of existing channels of distribution in such a way that established certification schemes would not be adversely affected.

Development of Differences in Yield between FX and Virus X-Infected Up-to-Date Potatoes.

*By J. G. Bald, M.Agr.Sc., Ph.D.**

Summary.

1. Two yield trials were conducted in which FX Up-to-Date potatoes were tested against certified Up-to-Dates containing virus X.

2. At first harvest, made while the plants were still very immature, there was no difference in total yield that could be attributed to the presence or absence of virus X.

3. At final harvest, the FX Up-to-Date yielded significantly higher than certified X-infected Up-to-Date.

4. It is concluded that, while the plants depend on current metabolism for the storage of food materials in the tubers, the tubers of FX and X-infected potatoes increase in total weight at approximately the same rate. Differences in yield begin to appear when the plants draw on the reserves of stored protein in the leaves for further expansion of the tubers.

1. Introduction.

A scheme has recently been evolved for the maintenance and multiplication of Up-to-Date seed stocks free from virus X and their distribution to farmers through certified seed organizations (Bald,

* An officer of the Division of Plant Industry.

1944*a*). To distinguish these stocks from commercial certified seed, which contains virus X, they have been termed FX seed stocks, and the same prefix will be applied to stocks of other varieties free from virus X as they are incorporated in the scheme. A series of yield trials has been planned to study the characteristics of the FX Up-to-Date stocks, which have, so far, been maintained in families, each a clone derived from a single virus-free tuber. As part of the plan, during 1943-44 two yield trials were laid down at the Council's Experimental Farm at Dickson, near Canberra. The trials included a comparison with a selected stock of certified Factor (Up-to-Date). The present paper is mainly concerned with the development of differences in yield between the FX and the certified, but X-infected, Up-to-Date potatoes. Other data were collected in these two trials, and other trials were made in co-operation with officers of the Tasmanian and New South Wales Departments of Agriculture and the Waite Agricultural Research Institute. These will be described later.

2. Yield Trial 1.

(i) *Description of the Trial.*

The first trial was designed as a lattice with sixteen treatments. The plots consisted of single chain-rows of 33 plants, accurately spaced. According to the design, there were five replications of each variety or clone. The distance between rows was a little more than 3 feet.

Of the sixteen treatments, twelve were allocated to twelve clones of FX Up-to-Date, one to a selected Up-to-Date stock from Tasmania, one to the certified Up-to-Date, and the other two to certified Brownell and Bismarck, both of which contained virus X.

The Tasmanian Up-to-Date was included on the assumption that it would contain virus X, but greenhouse tests revealed only about 8 per cent. infection. It may, therefore, be regarded as another FX stock. It was derived from single plant selections made in Tasmania by Dr. R. A. Scott, on the basis of yield, health, and quality (Bald, 1944*a*).

The seed tubers from the twelve FX Up-to-Date clones, the Brownell, and the Bismarck were from plots grown at the Tewkesbury Potato Station, Tasmania; the Tasmanian Up-to-Date was from Scottsdale, and the certified seed was from Crookwell, New South Wales. The parent crops were all grown in red basaltic loams, and the condition of the seed tubers at planting was uniform.

The trial was planted on 20th, 21st, and 22nd October, and fertilizer was applied by hand in the furrow. Although emergence was slightly irregular because of deep planting, there were less than 2 per cent. of misses, and early growth was exceptionally even and vigorous. As insufficient rain fell during the growing period, the trial was irrigated. After flowering, a severe infestation of potato moth threatened to destroy the trial, therefore the tubers were dug on 17th February while the plants were still very immature. Before digging was finished, a preliminary weighing of yields showed no differences between FX and X-infected Up-to-Dates. Half of one remaining block, including all selections and varieties, was left to mature. In addition, a small pilot trial, which had been planted in the same lattice design but with plots

of only two plants, was left. The plants were sprayed with phenothiazine to control the potato moth (Helson, 1944), and irrigated thoroughly. The tops of the plants in the pilot trial were cut off on 7th March, and the tubers were harvested a week later. The plants in the remaining half-block of the main trial had almost ripened off when the tops were killed by frost on 11th April.

The data recorded in this trial include—

(*a*) Leaf area rating of all plants (Bald, 1943*b*) made on 29th November, 5½ weeks after planting, on 17th December, after the inception of flowering, and on 13th January, after the majority of plants had reached their maximum size. At this stage, potato moth and hot, dry winds had caused some defoliation of the lower portions of the plants.

(*b*) Age rating of each plant on 17th December, according to whether the buds had unfolded at the tops of the main shoot, the plant was in flower, &c.

(*c*) Yield of tubers from 4½ main blocks dug prematurely. The tubers were graded into large, small, and infested with grubs of the potato moth or otherwise blemished. An estimate was obtained by sampling of the number of tubers in each category.

(*d*) Yields of the pilot trial on 7th March.

(*e*) Yields of the half-block dug at maturity.

In the present discussion, reference will be made only to total yields, and to some relevant sections of the leaf area data.

(ii) *Analysis of Yield, Preliminary Harvest.*

In Table 1 are the mean yields of the different selections and varieties. For analysis, the yields were converted to log values, because associations between leaf area, during the earliest stages of development, and yield were best revealed when the data were in that form.

TABLE 1.—PRELIMINARY HARVEST 14 TO 15 WEEKS AFTER EMERGENCE. (*Yields in pounds per chain row of 33 plants.*)

Variety or Clone.	Yield. Log Values. lb.	Yield. Antilog Values. lb.	Variety or Clone.	Yield. Log Values. lb.	Yield. Antilog Values. lb.
FX Up-to-Date 36	1·857	71·9	FX Up-to-Date 853 ..	1·819	65·9
101	1·837	68·7	910 ..	1·850	70·8
158	1·845	70·0	950 ..	1·808	64·3
166	1·833	68·1	Up-to-Date Tasmanian	1·827	67·1
224	1·810	64·6	Up-to-Date Certified	1·813	65·0
265	1·798	62·8	Brownell Certified ..	1·709	51·2
441	1·851	71·0	Bismarck Certified ..	1·863	73·0
597	1·844	69·8			
728	1·847	70·3	Significant difference	0·0456	

Among the FX Up-to-Date clones there were differences that reached the 5 per cent. level of significance, but the certified Up-to-Date was not significantly different from any of them. The mean for the twelve FX clones was 4·5 per cent. higher than for the certified Up-to-Date, and 1·5 per cent. higher than for the Tasmanian Up-to-Date. The yield of Bismarck was the highest of all varieties and clones, and of Brownell the lowest.

The leaf area data revealed that the differences between the FX clones were closely associated with differences in the first leaf area measurement made on 29th November; clones with a smaller leaf area at this stage yielded less. The differences in yield within the variety Up-to-Date could be eliminated by the use of the covariance of yield on the first leaf area measurement. Previous work (Bald, 1943*b*), and internal evidence from this experiment suggest that these differences were largely, or entirely, a reflection of differences in the dates at which the various clones and varieties emerged. Whether the rate of emergence of a clone is a permanent or temporary characteristic remains to be determined.

The regression of yield on the first leaf area measurement for Bismarck appeared to be of the same form as for Up-to-Date. The results in this experiment correspond with the general observation that Bismarck emerges very quickly after planting and has the same efficiency in the production of tubers as Up-to-Date. As will be seen from the results of the second and third harvests, however, the final yield was less than that of Up-to-Date because Bismarck is an early variety.

Brownell is not of the same type as the other two varieties. It takes longer to emerge from the soil, but from several years' observation, it appears to expand its first leaves somewhat more quickly. Thus, the regression yield on the first leaf area measurement was probably of a different form from that of Up-to-Date and Bismarck, and use of covariance to modify the yield totals may have over-compensated for the effect on yield of the late emergence. Even so, the modified yields for the first harvest were lower than those of Up-to-Date and Bismarck, i.e., Brownell was less efficient in the production of tubers during the earlier phases of development.

Table 2.—Yield as Percentage of the Mean for 12 Clones of FX Up-to-Date: Three Successive Harvests. Yield Trial 1.

Date of Harvest.	FX Up-to-Date 12 Clones.	Tasmanian Up-to-Date	Certified Up-to-Date.	Brownell.	Bismarck.
February 17	100	99	96	75	107
March 7	100	132	91	56	73
April 11	100	103	83	68	63

These points are summarized in the first line of figures in Table 2, where the mean yield of the 12 FX clones is taken as the standard, and the yields of the Tasmanian Up-to-Date, the Brownell, and the Bismarck are given as percentages of this value.

(iii) *Analysis of Yield, Second and Final Harvest.*

The pilot trial was planted beside the main trial at the same time and from the same lot of seed. The growth of the plants was in every way comparable, but as the plots consisted only of two plants the variability of the plot yields was much greater. However, the first leaf area rating gave a measure of differences in the date of emergence, which were the main cause of this variability. An analysis of covariance was made of yield on leaf area at this stage, and thus the trial was made to give reasonably accurate results.

Only the main conclusions bearing on the development of differences in yield will be outlined. Although the yield of the certified X-infected Up-to-Date was 9 per cent. lower than the average for the 12 FX clones, the difference was not significant. There were significant differences between FX clones, and at this time they were not associated with the leaf area during the first stages of growth. The Tasmanian Up-to-Date (Table 2) is of a type represented amongst the FX clones, the tubers of which developed rapidly between the first and second harvests. The Bismarck, being an early variety, had ripened off, and the Up-to-Date had outstripped it in yield. Brownell was still the lowest yielding of all. These results are illustrated in the second line of values in Table 2.

The yield figures for the final harvest from the remaining half-block of the main trial suffered the disadvantage that there was no true replication of varieties. On the other hand, the figures were derived from a split block in which the variability between varieties and clones in one half-block was largely reflected in the other half. Each lot of four adjacent plots, which represented a unit for the elimination of block differences in the lattice design, included three FX clones and one other variety. Lastly, there were ancillary data on leaf area, etc. In spite of the lack of replication, therefore, the last harvest can be made to give a good indication of final yields.

The simplest way of presenting the figures is as ratios between the yield of the later-harvested half-plot and the yield of the other half of the same plot. Such ratios are shown in Table 3, where the ratios for the Tasmanian Up-to-Date, the Brownell, and the Bismarck are compared with the mean ratio for the three FX clones in the same small block.

The ratios for FX clones are, on the whole, higher than the ratio for the certified X-infected stock. The ratio for the Tasmanian Up-to-Date, which contained only a small percentage of plants infected with virus X, is in agreement with those for the FX clones. In Block 3, the ratio for FX Up-to-Date is lower than in the other blocks, and also lower than the X-infected certified Up-to-Date, suggesting that the higher values for most of the FX clones might be the result of chance variation, or at least might not be a result of their freedom from

TABLE 3.—RATIOS OF YIELD AND LEAF AREA BETWEEN HALVES OF THE SAME PLOTS, ONE HALF HARVESTED ON THE 11TH APRIL (FINAL HARVEST), ANE THE OTHER ON THE 17TH FEBRUARY (PRELIMINARY HARVEST).

Ratios between Final and Preliminary Harvest.	Block 1.		Block 2.		Block 3.		Block 4.	
	FX U-to-D.	Bis-marck.	FX U-to-D.	Cert. U-to-D.	FX U-to-D.	Brown-ell.	FX U-to-D.	Tas. U-to-D
Yield	1·67	·95	1·61	1·37	1·27	1·43	1·57	1·58
Maximum leaf area	·97	·93	·95	·91	·72	·93	·97	·93

virus X. There is, however, an adequate explanation of this low value. The leaf area data show that in Block 3, although the plants in the two halves of the plots of the FX clones began growth at the same rate, the plants in the later-harvested portions did not attain the same size as those in the earlier-harvested portions. A slight change of slope in the ground where these half-plots were situated prevented them from receiving sufficient irrigation water, and this lack was reflected both in the size of the plants and the yield. Throughout the rest of this section of the trial, the half-plots were fairly similar. This may be seen from Table 2, where the ratios between the maximum leaf area attained by the late-harvested half-plots and the earlier-harvested portions are given below the yield ratios. The leaf area at this stage of development is very nearly proportional to the final yield. When the yield ratios are corrected for differences in leaf area, all the ratios for FX clones are higher than for the X-infected Up-to-Date. There is little doubt that in the later phases of growth the yield of FX clones increased more rapidly than the yield of the X-infected Up-to-Date, and that the final yields were higher. This point will be substantiated by the results of the second yield trial.

An estimate of the final yields in Yield Trial 1 is given in the last line of Table 2. The figures in Table 2 suggest that when $\frac{3}{5}$ of the final weight of tubers had been produced (February 17), there was little difference in yield between FX clones of Up-to-Date and the X-infected stock. Thereafter (March 7), clones of Up-to-Date showed differences in the rate of tuberation that were not necessarily reflected in the final yield (April 11). Infection with virus X depressed the rate of tuberation during the later stages of development, and therefore the final yield. The early variety, Bismarck, developed its tubers at least as rapidly as Up-to-Date, but the final yield was less because of the shorter growing period. In the earlier stages of tuberation, Brownell developed its tubers more slowly than Up-to-Date, but the difference was reduced towards the end of the growing period. The most legitimate comparison to illustrate this statement is between the Brownell and the certified Up-to-Date, both of which contained virus X.

3. Yield Trial 2.

(i) *Description of the Trial.*

The seed tubers for the second yield trial were derived from the Crookwell and New England districts of New South Wales. They included 8 of the 12 FX Up-to-Date clones (the other 4 were not available), the same stock of certified Up-to-Date as was used in the first yield trial, and the two varieties Katahdin and Sebago. The last two varieties were free from virus X. The trial was planted in randomized blocks with 6 replications, except that there were two plots of the certified Up-to-Date in each block, i.e. 12 plots in all. There were, thus, the equivalent of 12 treatments and 72 plots in the trial. As before, the plots consisted of chain-rows containing 33 plants.

The trial was planted on December 9 and 10, and, apart from some missing, grew vigorously and evenly. Sufficient irrigation water was applied to maintain growth. Attack by the potato moth was moderately severe at one stage, but infestation of the tubers was slight.

Leaf area measurements were taken on December 31, when the great majority of plants had emerged, and on January 19 at late flowering. In fact, very few flowers opened; in most instances the buds dropped off because of the hot, dry winds that were frequent at this stage of growth.

The preliminary harvest was dug on March 9th. Four plants in each plot were dug. To ensure that such small samples would be reasonably uniform, plants of approximately the same age were selected by using the plan of the trial on which the first leaf area ratings were recorded. The leaf area ratings ranged from 0 to 11. As far as possible, only plants rated 6 or 7 (Bald, 1943 *b*), with a plant growing on either side, were chosen. Any of these plants that were found on field examination not to have grown evenly, or to have suffered from disease or accident, were discarded, and others were substituted. After digging, the tubers were weighed and counted.

The tops of the remaining plants did not ripen off naturally; they were killed by the heavy frost of April 11th. The tubers were dug soon afterwards.

(ii) *Analysis of Yield, Preliminary and Final Harvest.*

The results of the preliminary harvest revealed differences between FX clones similar to those shown in the harvest of the pilot trial beside Yield Trial 1, but again the mean yield for these clones was not significantly different from that of the certified X-infected Up-to-Date.

A difference of 9 per cent, or more between the FX and X-infected Up-to-Date would have been significant. Choice of plants that had been similar in size on December 31st eliminated any differences in yield due to the unequal age of plants. On the other hand, it did not introduce an observable bias into this comparison. Although the Up-to-Date plants chosen were, on the whole, larger and probably higher-yielding than the average, the mean sizes of the FX and X-infected Up-to-Date plants were similar, and the selected plants were fair samples for comparison. The samples of Katahdin and Sebago,

however, were not comparable with the Up-to-Date samples. The plants of these two varieties emerged later, and by selecting those of the standard size, the largest and most vigorous were chosen for comparison with plants of Up-to-Date that were, for that variety, of intermediate size. The value for final yields of Katahdin and Sebago were actually less than those for the earlier harvest.

TABLE 4.—YIELDS IN POUNDS PER PLANT AND AS PERCENTAGES OF THE MEANS FOR FX CLONES OF UP-TO-DATE, PRELIMINARY AND FINAL HARVEST, YIELD TRIAL 2, CONSISTING OF SIX RANDOMIZED BLOCKS.

Date of Harvest.	Yield in—	Variety.			
		8 Clones FX Up-to-Date.	Certified Up-to-Date · 2 Replications.	Katahdin.	Sebago.
March 9	lb.	2·83	2·91	2·67	2·55
	%	100	103	94	90
April 11	lb.	3·33	2·95	2·02	2·25
	%	100	89	61	68

Standard deviation of the mean, March 9, 0·490 lb.
Standard deviation of the mean April 11, 0·337 lb.

The final yields clearly revealed a difference between the mean for the 8 FX clones and the mean for the X-infected Up-to-Date. Significant differences for this comparison were of the order of 6 per cent. or more, and there was, in fact, a difference of 11 per cent, between the two means. Yields for Katahdin and Sebago were about two-thirds that for the Up-to-Date. However, the comparison did not truly indicate the relative value of the varieties, as the proportion of No. 1 grade tubers of Katahdin and Sebago was higher than of Up-to-Date, and this somewhat reduced the margin between them.

As the foliage of the plants in this yield trial was killed by frost while it was still green, the final yields do not represent the yields of a fully mature crop. The available evidence, e.g., Table 2, suggests that the difference between FX and X-infected Up-to-Dates would have been greater, if the plants had been allowed to ripen off. As happened with Brownell in the first yield trial, the Sebago, and possibly the Katahdin, might have gained in yield relatively to Up-to-Date.

4. Discussion.

In the comparison between FX and X-infected Up-to-Date, the inclusion of 8 or 12 clones guarded against the possibility that the development of the difference in yield might be due to agronomic

differences and not to the effects of infection. The 12 FX clones were derived from mass-selected stock of certified Up-to-Date and there seems to be no reason why they should not be accepted as agronomically a fair sample of that stock. The certified X-infected stock used in the trial had been maintained for many years merely by roguing diseased and off-type plants, and almost certainly consisted of a number of clonal forms. Comparison between the FX and certified stocks is, therefore, justifiable.

It seems fair to conclude that the differences in yield between the two stocks of Up-to-Date were almost entirely due to the presence of virus in the certified stock. During the stages of growth when the plants were developing tubers mainly by the utilization of currently-formed metabolites, no significant differences in yield were evident between the FX clones taken together and X-infected plants. When the plants began to draw on the reserve foodstuffs stored in the foliage, in order to complete the growth of their tubers, real differences in yield became evident. The substitution of virus protein for the normal chromoprotein (chloroprotein) in diseased plants (Woods and Du Buy, 1941), provides a reasonable explanation of their depleted reserves.

Implied in this explanation is the hypothesis that the amount of labile protein available for translocation to the tubers, rather than the amount of the main storage materials, the carbohydrates, potentially sets a limit to the yield. In former publications, a brief discussion of these ideas was given (Bald, 1942, 1943 *a*). The results of the experiments described in this paper give some further support to the hypothesis, and examination of the available literature on the biochemical changes in potato plants infected with mosaic diseases also reveals data that support it.

Two papers that contain results particularly applicable to this problem are those of Cockerham (1939) and Stone (1936). The former deals with the metabolism of healthy and X-infected President potatoes. One of the main conclusions is that "the *gross* levels of the carbohydrates with regard to seasonal changes in level, and the *gross* trend of diurnal and seasonal variation are closely similar in both healthy and diseased material". This, and the facts that under Australian conditions infection with virus X does not affect either the leaf area up to the time it reaches its maximum (Bald, 1943 *a*) or the yield until tuberation is well advanced, together indicate that infection with virus X causes no serious loss of efficiency, either in photosynthesis or the translocation of the products of photosynthesis.

Small differences which were shown to exist in the diurnal fluctuations of starch, sucrose, and reducing sugars (Cockerham, 1939) might well be explained by differences in the rhythm of virus and chromoprotein synthesis, and a corresponding difference in incidence of the demand for energy-producing carbohydrates. There is an indication of such a difference in incidence, e.g., in the nitrogen metabolism of healthy potatoes and of potatoes infected with leaf roll virus (Barton-Wright and McBain, 1933).

The main difference in composition between healthy and X-diseased tissues is a slight but significant increase in total nitrogen with infection, a difference which increases with increasing age of the plant until

the presenescent period begins. Also, in the presenescent period, there is a slightly higher concentration of carbohydrates in the leaf lamina of diseased plants, and a significantly lower concentration in the petiole. The higher nitrogen is almost certainly due to the presence of virus protein. The difference in carbohydrate levels is attributed to the "diminished utilization of carbohydrates in the diseased plants" According to the present hypothesis, the diminished utilization of carbohydrates would be linked with diminished supplies of reserve protein which result in a reduction in the rate of storage of reserve materials in the tubers during the later stages of their development.

Infection with virus X is also accompanied "by a reduction in the number of flower trusses, and a reduction in the number of flowers per truss". President flowers more freely than most varieties, and flowering makes sudden demands on the mobile protein of the plant, particularly as it often coincides with the formation of the first tuber rudiments. The inability of the plant to hydrolyse and resynthesize the virus protein offers a sufficient explanation of this effect.

In infection with mosaic viruses causing more severe symptoms on potato than virus X, both growth of the tops and yield are affected. In an American experiment on plants of the variety Green Mountain, carrying what was probably infection with two viruses—X and A or Y type viruses (Stone, 1936), painstaking day-to-day measurements of leaf area were made on four mosaic plants and one healthy plant (probably carrying virus X). Analyses were made in the senescent stage, after the leaf measurements were concluded, of the starch, sugar, total carbon, and ash. Mosaic infection had little effect on the number of leaves produced, but the leaves on the infected plants were smaller than those on the healthy plant and had not the same orderly sequence of expansion. Hence the total leaf area was smaller. Sugar and starch content of the leaves on a dry weight basis were lower than in the normal plant, but the carbon content was not very different. The ash was not significantly different. The yield, both absolute and on a leaf area basis, was smaller, showing that infection reduced the efficiency of diseased plants, per unit leaf area, during the experimental period. This point is discussed more fully elsewhere (Bald, 1944 *b*). The normal ash content of diseased plants suggests that the roots were functioning normally, and the small differences in total carbon that the photosynthetic mechanism was functioning, if not at full efficiency, at least better than the reduced amounts of starch and sugar would suggest. The presence of unhydrolysable virus was probably a limiting factor in the expansion of the leaves, as well as in the growth of the tubers. That the amount of hydrolysable protein, rather than the amount of available carbohydrate, was responsible for the reduction in yield of tubers per unit of leaf area, is suggested by equal amounts of carbon on a dry weight basis in the tubers, and the larger proportion of the total sugar and starch left in the senescent tops of mosaic-diseased plants. In spite of the equality of total carbon, there was a smaller proportion of sugar and starch in the tubers, as in other portions of the infected plants. If this were explained by difficulties of translocation, the argument that the presence of virus protein directly caused the reduction in yield would be weakened, but it has not been proved that translocation was less efficient than in normal plants.

Alternatively, it may be suggested that some of the starch and sugars were used in the local multiplication of virus protein that occurs in developing tissues. That the translocation of some materials other than carbohydrates was not affected is suggested by the ash content of mosaic tubers, as well as by the total carbon, both of which were equal to, or higher than, the content of healthy tubers.

Similar evidence may be adduced from other papers describing the physiological effects of virus diseases on their host plants. However, it would be unwise to press such evidence too far, because much of it is liable to more than one interpretation. With this reservation it may be claimed that there are now good grounds for assuming that the yield of tubers depends at least as much on the protein metabolism of the potato plant as on the carbohydrate metabolism, and that the amount of labile protein in the plant is more likely to limit the yield of plants infected with mosaic diseases than the amount of available carbohydrate.

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C.10595/44.—

The Permanence of Australian Vertical Retort Creosote Oils.

Correlation of Boiling Range with Leaching Losses, Evaporation Losses, and Changes in Composition.

*By David E. Bland, M.Sc.**

Summary.

Three Australian vertical retort creosotes of low, medium, and high creosote boiling range have been subjected to a permanence study. A number of blocks of *Doryphora sassafras* were impregnated with each of the creosotes, one set was exposed to leaching only, one set to evaporation and oxidation only, and one set was protected completely by sealing in airtight tins. Blocks treated with each creosote were withdrawn from each set from time to time and the creosote extracted and examined.

The high boiling creosote showed only small loss by evaporation, but lost considerably by leaching. It proved most permanent as its losses both by leaching and by evaporation were smaller than those from either of the two lighter creosotes. In general, loss of creosote took place rapidly in the first three months of exposure and at a much reduced rate thereafter.

The only notable change in composition was the drop in tar acid content, which was most marked in the leaching specimens, greatest from the low boiling creosote, and least from the high boiling creosote. Laboratory experiments indicate that leaching does not entirely remove the tar acids but causes them to approach a terminal concentration which is little affected by further leaching. The decrease in tar acid content of creosote in evaporation specimens and the appearance of a fraction not extractable by benzene, indicates that some oxidation of creosote takes place in the wood, resulting in the deposition of a benzene-insoluble residue derived from the tar acids. This accounts for only a small proportion of the tar acids.

The toxicity of creosotes is decreased both by exposure to leaching and by evaporation and oxidation.

It is concluded that a satisfactory degree of permanence and permanence of toxicity can be expected from Australian vertical retort creosotes provided that low boiling creosote is avoided.

1. Introduction.

The rapidly dwindling supplies of durable timbers in Australia make it highly probable that adequately treated non-durable species will become increasingly important for use in situations where conditions favour decay or insect attack. Creosote oil, which is the most widely used wood preservative throughout the world, will probably be largely depended upon for this purpose. The assessment of Australian creosotes as wood preservatives is therefore a problem fundamental to the development of any large-scale creosoting industry in this country. They are of a type for which practically no data are available among the reports of either experimental or large-scale creosoting operations.

* An officer of the Division of Forest Products.

There appear to be only three types of method for assessing the permanence of wood preserving oils. One method which has been extensively employed, no doubt because it gives results quickly, consists in the examination of treated wood which has been in service for long periods of time. The amount and nature of the creosote present is determined by extraction and analysis of the extracted creosote. These data alone are of little value unless they can be compared with the analytical data on the oil used in the treatment, and with data on the retention of oil obtained in the treatment. The weakness of this method is that the treatment may have been carried out with no particular attention to the preservation of data concerning the creosote, the treatment, or the retention obtained, so that the bases of comparison must sometimes rest upon questionable assumptions. Also, where major changes in industry have occurred, the type of creosote examined may be quite different from the types now being produced, so that the data obtained may have no direct application to creosotes now available. In any case, the opportunities for the application of this technique in Australia are few, because there has been no large-scale use of impregnated timber here.

A second method of approach, which has been employed, aims at the creation in the laboratory of conditions which bring about all those chemical and physical changes resulting from weathering, in as many days as actual weathering takes years to induce. These methods have not come into any general use because of the impossibility of any accurate correlation between the results of these tests and performance of a creosote in the field. There is no certainty that the artificially created conditions have accelerated any one element of change, say oxidation, to the same extent as any other element, say leaching.

A third possible method would consist in the treatment of timber specimens with carefully analysed creosotes, noting the retention of creosote. These treated specimens could then be exposed at chosen sites, and examined at regular intervals. The results obtained in this way would be accurate and apply to readily available creosotes. The accumulation of data in this way is a laborious process; it has, however, been followed in the present investigation as the only reliable method, and has yielded valuable data in three years. This is a short space of time compared with the life expected from treated timber, but nevertheless the relative merits of different creosotes have been clearly disclosed.

In earlier tests on the leaching of creosote conducted by the Division of Forest Products, lengths of timber were impregnated with the creosote under test and the treated timber immersed in water. The timber was then withdrawn from time to time, portions sawn off, and their creosote contents determined. Difficulties encountered with this method were that end-leaching losses were serious and extended many inches down the piece and that the distribution of creosote along the test timber, even before exposure, was found to be far from uniform. In addition, insufficient creosote was obtained from the cut-off sample for the standard tests to be carried out. On the basis of these trials, it was decided that further tests would be conducted with a large number of individual test pieces, the creosote content of each one of which was accurately

known. The test pieces have been made such a size as to give, on extraction, sufficient creosote for the carrying out of standard tests. The characteristics of the creosotes used were determined and recorded.

2. Creosotes Tested.

Creosotes, A, B, and C, the main analytical characteristics of which are given in Table 1, were tested under identical conditions. These creosotes have already been subjected to an exhaustive toximetric study, reported in an earlier paper*.

TABLE 1.—CHARACTERISTICS OF CREOSOTES USED IN TESTS.

Creosote.	A.	B.	C.
S.G. 38°/20° C.	0·948	0·963	0·982
Standard distillation—			
0–205° C. per cent.	5·7	0·8	1·4
0–230° C. per cent.	39·8	2·5	2·5
0–315° C. per cent.	91·5	55·1	20·2
Composition—			
Tar acids per cent.	25·9	17·0	16·9
Tar bases per cent.	4·0	3·8	4·2
Unsaturated hydrocarbons per cent. ..	5·0	8·0	10·0
Aromatic hydrocarbons per cent. ..	33·5	31·2	29·3
Paraffins and naphthenes per cent. ..	31·6	40·0	59·6
Viscosity—centipoise—			
25° C.	5·2	15·8	Semi-solid
70° C.	1·8	3·6	7·4
Surface tension—dynes/cm.—			
25° C.	31·3	30·5	..

3. Outline of Experiment.

The timber used throughout the test was sassafras (*Doryphora sassafras*) all cut from one tree. The boards of nominal 6 in. by 1 in. size, were kiln dried until each board showed a moisture content of 13 per cent., as determined by the blinker moisture meter. They were then dressed to 5½ in. by ⅞ in., and cut into 12-in. lengths, with a 1-in. moisture content disk between each. The moisture content of each disk was determined by drying at 105°C. for 24 hours. The moisture content of each board was taken as the mean of the two disks at its ends; it proved to be very uniform throughout the test timber. All test boards were then marked by punching deeply into the end of each board a number denoting the creosote, a number denoting the proposed time of examination, and a number distinguishing it from its duplicate.

All test boards were weighed to the nearest gram, treated with the appropriate creosote by the open tank method, allowed to drain and dry, and reweighed. The treating schedule was two hours in the hot bath at 200°F., followed by removal to the cold bath, where the boards

* Bland, D. E. (1942).—*J. Coun. Sci. Ind. Res.* (Aust.) 15: 135.

remained overnight. As soon as they were dry, the boards were given a thick end-coating of "Durofix." Immediately after the treatment, six boards, two treated with each of one of the three creosotes, were examined as described in Section 4 below.

The remaining treated boards were divided into three groups. One-third of those treated with each creosote were placed in a well-fitting sheet metal box, and the lid soldered to make an airtight joint. One-third were stacked in a basement in the style of a seasoning stack, allowing free circulation of the air round each board. These boards were thus protected from the weather but subject to evaporation losses. The remaining third were placed in a frame which was suspended under a wharf in a river. The construction of the frame was such that the boards were about an inch apart and held only by the end $\frac{1}{4}$ in. so that the water circulated freely around each board.

A series of test boards, consisting of boards treated with each creosote from the sealed, from the evaporation, and from the leaching set, were withdrawn at the end of 3 months, 6 months, 1 year, 2 years, and 3 years, and examined as described below. Examinations were made more frequently at first, on the assumption that the rate of loss would decrease with time.

Creosotes A and B were subjected to water extraction in the laboratory. The device used is shown in Fig. 1. It consists of a container 4 in. in diameter and 30 in. high. From the bottom of the container a $\frac{3}{8}$-in. copper overflow tube runs to a height of 20 inches, where it bends over, so that outlet is directed downwards. The overflow tube is to maintain a constant level of liquid in the container. An S-shaped tube is soldered to the top of the container; by connecting the lower end of this to a water supply, water may be caused to drip down the centre of the container.

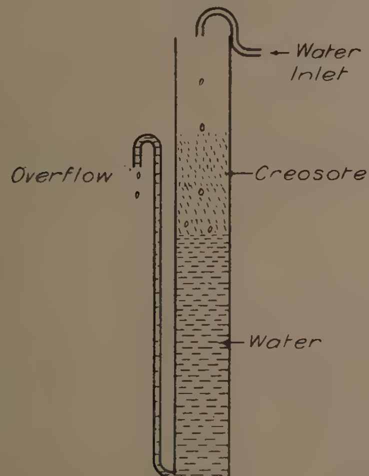

FIG. 1.—Apparatus for laboratory water-leaching of creosote.

In order to carry out an extraction, water was poured into the container until it flowed out of the overflow tube, and one litre of the creosote to be tested was poured on top of the water. This caused about a litre of water to flow out of the overflow tube. Water was then allowed to drip through the creosote at the rate of three litres per hour for one week, and, at the end of this time, the creosote was separated from the water in a separating funnel. This test can, of course, be applied only to creosotes which float on water and which are liquid at ordinary temperatures. After the extraction, the distillation curve, chemical analysis, and toxicity of the creosote were determined.

4. Details of Examination of Test Boards.

The test boards when withdrawn from exposure were wiped clean and weighed to the nearest gram. Those from the leaching set were stored in a tin of water until immediately before testing.

The board under test was cut into ¼-in. cross-grain slices. All chips were carefully collected and placed in a large sheet metal separating funnel with a fine wire gauze strainer near the apex of the cone. The chips were firmly pressed down and covered with a commercial grade of benzene, the funnel being sharply tapped to dislodge the last of the entrapped air from the chips; it was then allowed to stand overnight. The benzene solution was run off and filtered into a tared distilling flask and distilled to 150°C. The chips in the funnel meanwhile were again covered with fresh benzene, which was allowed to stand for two hours. The creosote in the flask was allowed to cool and the second benzene extract added and the benzene distilled off as before and so on until the extraction of the creosote was complete. By this method of cold soaking of thin-cross-grain slices it was found that the creosote was almost completely extracted by three changes of benzene. Control boards were extracted in this way immediately after treatment, and the recovery of creosote was 97·3 per cent. of Creosote A, 97·7 per cent. of Creosote B, and 97·5 per cent. of Creosote C. A sample of chips yielded no further creosote on extracting in a soxhlet extractor. The specific gravity, toxicity, distillation range, and analysis of the creosotes were determined by the methods described previously (Bland, loc. cit.). The specific gravity and toxicity were determined on the creosote as extracted, and the analysis was made on the distillate from the distillation test.

The chips from several boards were oven-dried and weighed and the weight compared with the calculated oven-dry weight of the test board. In the case of the leaching specimens the water absorption was assessed as: Wet weight — (calculated oven-dry weight + weight of creosote recovered) In two cases test boards were split into a shell and core by sawing off the outside ½-in. shell and extracting the shell and core separately. The extracted chips were oven-dried, and the amounts of creosote in the shell and in the core expressed as percentages of the weight of oven-dry wood. The tar acid contents of the creosotes from the shell and from the core were determined.

5. Results.

(i) *Results of Treatment.*

In all, 44 boards were treated with each creosote. The mean oven-dry weight of boards treated with creosote A was 600 g.; of those treated with creosote B, 625 g.; of those treated with creosote C, 625 g. The mean retentions were 424 g., 401 g., and 378 g. of A, B, and C, respectively.

(ii) *Losses of Creosotes.*

As would be expected, there was no loss from the sealed boards. All estimates of the quantity of creosote present made by unsealing the boards and weighing were within 1 per cent. of the original quantity. At each examination up to two years one sealed board was sliced and extracted; the recovery of creosote was always within 3 per cent. of the original. This almost quantitative recovery confirms that there is no combination between the creosote and the wood substance. The sealed boards were not further examined after two years.

The losses of creosotes by evaporation are plotted against months of exposure in Fig. 2. The light creosote A showed a 16·1 per cent. loss during the first three months and continued to lose, but at a much reduced rate, as shown by the slope of the curve. The medium creosote B showed a 6·2 per cent. loss in the first three months and some further loss thereafter. The heavy creosote C showed a 3·0 per cent. loss in three months and no significant loss thereafter.

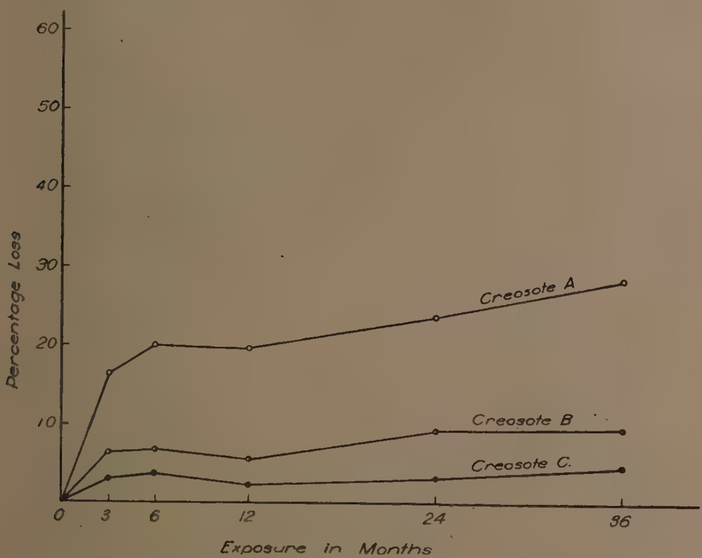

FIG. 2.—Losses of creosotes by evaporation.

The creosote content of the evaporation specimens was checked by direct weighing of the test boards. The amount of creosote recovered by extraction was almost always less than that estimated to be present from the weight of the board. The quantity of this "unaccounted for creosote" was very variable within the range of 0 to 25 g., with one of 50 g.; no general increase in its amount over the time of exposure could be traced. This is not surprising in view of the fact that it could be estimated only as a small difference between two relatively large numbers, neither of which could be measured with great accuracy. There was, however, a significant difference in amounts unaccounted for between the three creosotes tested. For the six examinations covering three years of exposure the mean amount of unaccounted for creosote, expressed as percentage of the creosote originally present, was 5·3 per cent. for A, 2·1 per cent. for B, and 1·1 per cent. for C. Unfortunately, the dry weight of chips after extraction has been recorded in a few cases only. These few were, however, 0 to 30 g. heavier than the calculated oven-dry weights of the test boards. The significance of these facts is discussed in Section 6. The losses by leaching are similarly shown in Fig. 3. Here, the losses are in the same order as the evaporation losses; greatest from A, least from C. The loss of light creosote A was 21·5 per cent. in three months, it has risen steadily to 55·0 per cent. in three years and appears to be continuing. Medium creosote B lost 15·4 per cent. in three months and 33·8 per cent. in three years. Creosote C suffered a loss of 18·5 per cent. in three months and 20·8 per cent. in three years.

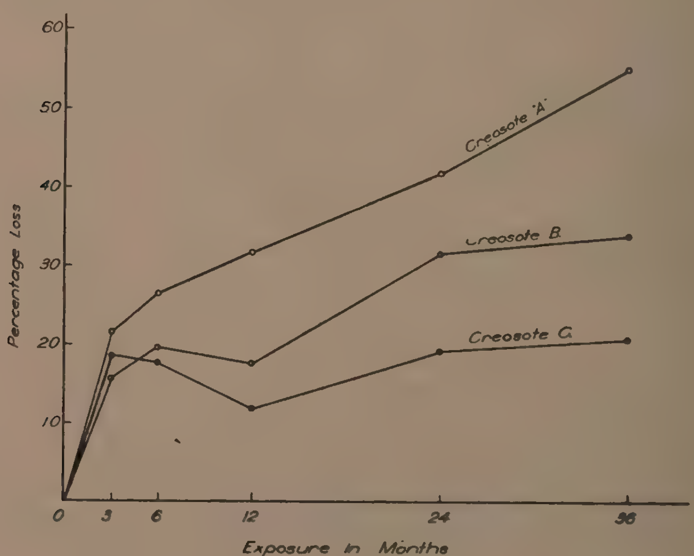

FIG. 3.—Losses of creosotes by leaching.

It may be seen from the figures in Table 2 where the creosote and water contents of the leaching specimens are given as percentages of weight of oven-dry wood, that uptake of water occurred concurrently with loss of creosote. From three months onwards the total liquid (creosote and water) held by the wood remained roughly constant at 120 per cent. of the oven-dry weight.

TABLE 2.—CREOSOTE AND WATER CONTENTS OF LEACHING SPECIMENS.

Creosote.	Time of Exposure (Months).	Creosote Content as Percentage of Oven-dry Weight of Board.	Water Content as Percentage of Oven-dry Weight of Board.	Creosote + Water Percentage Oven-dry Weight of Board.
A	0	70·7	13·0	83·7
	3	54·0	60·3	114·3
	6	50·2	69·0	119·2
	12	48·8	81·0	129·8
	24	37·1	94·1	131·2
	36	33·0	104·0	137·0
B	0	64·2	13·0	77·2
	3	61·5	60·0	121·5
	6	51·1	67·0	118·1
	12	48·8	71·1	119·9
	24	45·1	84·6	129·7
	36	41·1	71·0	112·1
C	0	60·4	13·0	73·4
	3	58·0	56·0	114·0
	6	51·1	63·9	115·0
	12	49·0	81·0	130·0
	24	44·1	83·7	127·8
	36	42·6	76·5	119·1

Two leaching specimens which had been exposed for two years were split into a shell and core. The first board which originally retained creosote A to the extent of 58·7 per cent. of its oven-dry weight was found to retain in the outside $\frac{1}{8}$-in. shell 20·6 per cent. and in the core 40·9 per cent. The creosote from the shell contained 6·5 per cent. tar acids and that from the core, 9·6 per cent. tar acids. The second board, which originally retained 66·1 per cent. of creosote B, gave from the shell 36·5 per cent. and from the core 52·8 per cent. of creosote which contained 8·1 and 12·4 per cent. of tar acids respectively.

(iii) *Effect of Exposure on the Distillation Ranges of the Creosotes.*

The distillation curves, determined as described previously (Bland, loc. cit.), of creosote A as received, as extracted from control boards immediately after treatment, as extracted from the leaching specimen after three years, and as extracted from the evaporation specimen after three years, are shown in Fig. 4. The lower position of the curve of the creosote from the control shows some loss of the more volatile fractions during the treatment. The curve of the oil from the evaporation specimen falls very much below that of the control thus confirming that considerable loss of the more volatile fractions of creosote occurs by evaporation from treated wood. The curve for the oil from the leaching

specimen falls still further below the control curve showing that, for creosote A, loss of the volatile fractions occurred to a greater extent by leaching than by evaporation.

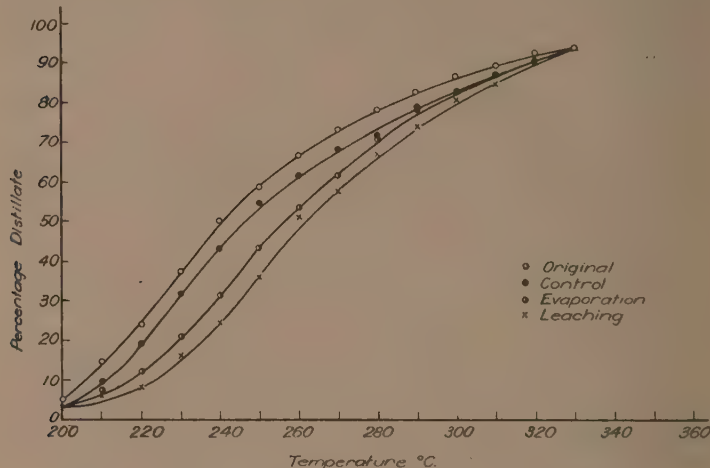

FIG. 4.—Effect of exposure on distillation curve of creosote A.

Similar data for creosote B disclose only small loss of its more volatile fractions during the treatment and some further loss during three years of exposure. For example, the original creosote yielded 54 per cent. distilling to 315°C., that from the control board 52 per cent., and those extracted after three years of exposure 48 per cent. The distillation curves of the creosotes from the leaching and from the evaporation specimens coincided almost exactly.

The curves for creosote C could not be determined with the same accuracy as those for the two lighter creosotes A and B. In the lower temperature range (below 300°C.) the small amount of distillate made it difficult to maintain the standard distillation rate and take readings of the rapidly rising temperature, whereas above 320°C. solidification of distillate in the condenser and difficulty in maintaining steady distillation of this high boiling material made for decreased accuracy.

The results in general indicated slight loss of volatiles during the treatment, and no further loss during exposure.

(iv) *Changes in Composition.*

Analyses of creosotes extracted, after different periods of exposure, from evaporation and from leaching specimens are shown in Tables 3 and 4 respectively. From these results it is apparent that the only major changes in composition which followed exposure were the decreases in tar acids and unsaturated hydrocarbons. It is noteworthy that all three creosotes extracted from boards immediately after the treatment contained less tar acids than the original creosotes.

In the evaporation specimens the tar acid content of A underwent a steady decrease, likewise that of creosote B. The rather erratic variation shown by creosote C is probably due only to analytical

difficulty caused by the thick sticky nature of its tar acids which were troublesome to separate cleanly and to float and measure with accuracy. The loss of tar acids from the leaching specimens was greater than that from the evaporation specimens. The tar acid content of creosote A has undergone a marked steady drop over the three years, that of B has undergone a smaller drop, and C has shown no significant decrease. The tar bases appear to have been scarcely affected. The aromatic and saturated hydrocarbons show a rise in percentage with increasing time of exposure due, of course, to the removal of other constituents.

TABLE 3.—ANALYSES OF CREOSOTES EXTRACTED FROM EVAPORATION SPECIMENS.

Creosote.	Constituent.	Time of Exposure (Months).					
		0.	3.	6.	12.	24.	36.
A ..	Tar acids	21·0	18·8	18·0	17·4	16·7	16·0
	Tar bases	4·1	4·6	4·4	2·8	4·5	3·9
	Unsaturated hydrocarbons ..	6·3	5·3	5·6	4·2	4·0	3·0
	Aromatic hydrocarbons, paraffins, and naphthenes ..	68·6	71·3	72·0	75·6	74·8	77·1
B ..	Tar acids	15·0	14·7	15·1	13·1	13·0	11·8
	Tar bases	5·3	5·3	4·8	5·1	4·5	5·0
	Unsaturated H.C.	6·5	7·6	6·9	6·7	4·5	1·1
	Aromatic H.C., paraffins, and naphthenes	73·2	72·4	73·2	75·1	78·0	82·1
C ..	Tar acids	14·2	14·5	16·9	14·4	16·0	15·0
	Tar bases	5·5	5·1	4·6	5·0	5·0	4·4
	Unsaturated H.C.	11·0	9·4	9·6	5·3	5·5	3·0
	Aromatic H.C., paraffins, and naphthenes	69·3	71·0	68·9	75·3	73·5	77·6

TABLE 4.—ANALYSES OF CREOSOTES EXTRACTED FROM LEACHING SPECIMENS.

Creosote.	Constituent.	Time of Exposure (Months).					
		0.	3.	6.	12.	24.	36.
A ..	Tar acids	21·0	18·0	14·6	12·3	8·5	8·0
	Tar bases	4·1	3·8	3·7	3·5	3·1	2·5
	Unsaturated H.C.	6·3	5·3	5·6	5·1	3·9	2·3
	Aromatic H.C., paraffins, and naphthenes	68·6	72·9	76·1	79·1	84·5	87·2
B ..	Tar acids	15·0	15·0	12·5	11·7	9·8	10·3
	Tar bases	5·3	5·0	4·6	4·4	4·0	4·6
	Unsaturated H.C.	6·5	7·7	7·9	5·8	6·0	3·0
	Aromatic H.C., paraffins, and naphthenes	73·2	72·3	75·0	78·1	80·2	82·1
C ..	Tar acids	14·2	15·2	13·8	12·9	14·3	12·3
	Tar bases	5·5	4·0	4·6	4·7	4·9	4·3
	Unsaturated H.C.	11·0	7·3	8·0	5·4	3·0	5·0
	Aromatic H.C., paraffins, and naphthenes	69·3	73·5	73·6	77·0	77·8	78·4

(v) *Effect of Exposure on the Specific Gravity.*

S.G. 38°/20°C. of the three creosotes after different times of leaching and evaporation are shown in Table 5. Creosote A showed an increase in S.G. during the treatment, followed by a steady drop consequent upon leaching. The effect of evaporation (and oxidation) is not so simple; it appears to have caused a preliminary drop followed by a rise. This may be the result of opposing effects of evaporation and oxidation which are discussed in Section 6. Creosote B showed a steady increase of S.G. in the evaporation specimens, whereas in the leaching specimens it has dropped by 0·007 in the first six months and then remained constant. The S.G. of creosote C has remained constant in the leaching specimens but has increased steadily in the evaporation specimens.

TABLE 5.—EFFECT OF EXPOSURE ON S.G. OF CREOSOTES.

(S.G. 38°/20°.)

Nature of Exposure.	Creosote.	Time of Exposure (Months).					
		Original Creosote.	0.	6.	12.	24.	36.
Leaching	A ..	0·948	0·956	0·932	0·928	0·924	0·920
	B ..	0·963	0·959	0·952	0·953	0·952	0·953
	C ..	0·982	0·982	0·982	0·981	0·983	0·982
Evaporation ..	A ..	0·948	0·956	0·943	0·942	0·947	0·950
	B ..	0·963	0·959	0·959	0·961	0·964	0·970
	C ..	0·982	0·982	0·987	0·989	0·991	0·996

(vi) *Effect of Exposure on Toxicity.*

It was originally intended to determine both inhibiting and killing concentrations for three test fungi for all creosotes at all examinations, but because of pressure of other work the toxicity determinations had to be omitted from the examinations at two and three years.

Table 6 shows the killing concentrations determined for the original creosotes, for the creosotes from the control boards, and for the creosotes after 3, 6, and 12 months' exposure. Inhibiting concentrations were also determined, but these varied quite unsystematically within a given range for each creosote. The indefinite nature of inhibiting concentration was discussed fully by Bland (loc. cit.). It may be seen from Table 6 that the following results hold generally, but not in every case. The toxicity of creosotes extracted from control boards immediately after treatment was less than the toxicity of the original creosote. The toxicity of the creosotes in both the evaporation and leaching specimens decreased, but not seriously, during the first twelve months of exposure. Creosote A, which was most toxic originally, remained the most toxic at the end of twelve months' exposure.

TABLE 6.—TOXICITY OF CREOSOTES AFTER EXPOSURE.

(*Killing concentrations: per cent. in agar medium.*)

Test Fungus.	Creosote.	Original Creosote.	Creosote from Control Board.	From Evaporation Specimens.			From Leaching Specimens.		
				3 mths.	6 mths.	12 mths.	3 mths.	6 mths.	12 mths.
Lentinus lepideus	A	0·3	0·4	0·3	0·6	1·0	0·6	0·6	2·0
	B	1·0	2·0	2·0	3·0	over 3·0	3·0	2·0	3·0
	C	1·0	2·0	0·6	over 3·0	over 3·0	over 3·0	over 3·0	over 3·0
Polystictus versicolor	A	0·08	0·1	0·15	0·1	0·1	0·2	0·1	0·2
	B	0·6	0·3	0·4	0·3	0·6	0·3	0·3	0·4
	C	1·0	1·0	over 3·0	2·0	3·0	2·0	2·0	3·0
Madison 517 ..	A	0·06	0·1	0·1	0·06	0·15	0·3	0·08	0·3
	B	0·2	0·2	0·1	0·2	0·6	0·15	0·15	0·5
	C	0·3	0·6	2·0	0·6	over 3·0	0·6	over 3·0	3·0

(vii) *Water Leaching of Creosotes in the Laboratory.*

Creosote A was subject to water leaching in the laboratory with results very similar to the results of its exposure to leaching as the impregnant of wood. The distillation curve was affected in just the same way as the creosote in the leaching specimen (Fig. 4). The tar acid content dropped from the original 25·9 per cent. to 6·3 per cent. There was no other important change in chemical composition. On leaching some of this creosote for a second week the tar acid content dropped to 5·2 per cent.

In these laboratory tests it was possible to leach quantities of the creosote sufficient to yield, on separation, enough tar acids for a standard distillation test. The results of distillation tests on tar acids separated from the original creosote, and tar acids separated from the creosote after leaching, are shown in Fig. 5. It is clear from these curves that the effect of leaching has been to remove the low boiling tar acids. For example, the original tar acids contained 61 per cent. distilling below 230°C. whereas the acids from the leached creosote contained only 17 per cent. The curve for the neutral oil from the leached creosote fell about 1 per cent. below the curve for the neutral oil from the original creosote, thus showing the neutral oil to be practically unaffected by the leaching. Creosote B was subject to the same test, with the result that the tar acid content fell from 17·0 per cent. to 8·1 per cent.

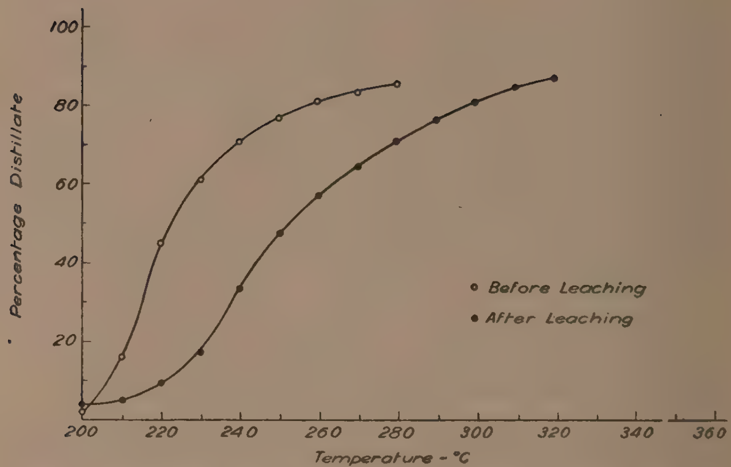

FIG. 5.—Distillation curves of tar acids from creosote A before and after leaching.

In these tests the decrease in volume of the oil was far greater than corresponded to the loss of tar acids. This was due to mechanical loss, for when water from the overflow tube was collected and examined closely it was seen to contain droplets of oil only just visible to the naked eye.

The toxicity of the water-leached creosotes was determined in the usual way. The killing concentrations for A were, for the three fungi in the order given in Table 6: over 3·0, 0·3, 0·3, and for creosote B: over 3·0, 0·3, and 1·0. Comparison of these figures with those in the last column of Table 6 shows that the decrease in toxicity following the laboratory water-leaching test was greater than that caused by three years of leaching from the test boards.

6. Discussion and Conclusions.

In planning this experiment it was considered that, as creosoted timber may be employed in a multitude of different situations, each having its own particular set of conditions affecting the life of the timber, any attempts at the accumulation of data by testing creosotes at numerous situations—having equally numerous combinations of the variables—could give only fragmentary data as the result of much labour. By means of exposure in certain ideal situations, it was sought to evaluate separately the effects of leaching, evaporation, and combination with the wood substance.

Creosote in service may become ineffective in three ways: it may be lost from the wood by evaporation; it may be lost by leaching; it may combine with the wood substance to form a non-toxic compound. The term loss is here used to denote loss of the creosote as a whole or merely loss of its toxic constituents, which would have the same result. The almost quantitative recoveries of creosote from the sealed specimens show that combination with wood substance does not occur to any

appreciable extent and therefore need not be considered. Loss by evaporation and leaching and also loss of the most toxic constituent, the tar acids, all take place.

The different retentions obtained from the three creosotes with the same treating schedule are quite in keeping with their physical properties. Their surface tensions (T) being approximately the same, their penetrativities (T/η) depend upon their viscosities (η). The retentions obtained were in fact in the reverse order of their viscosities. This difference would probably not be of any practical importance because it could be overcome by varying the treating schedule to give the required retention. The absorptions obtained in the treatment of the test boards were much heavier than those used in practice, and it may be that the heavier treatments tend to exaggerate the losses. That is, the percentage loss from a board impregnated with creosote to the extent of 50 per cent. of its oven-dry weight may be greater than from a similar board containing 30 per cent. creosote. These heavy treatments were, however, necessitated by the demands of the experiment, namely that the boards should be of a size amenable to manipulation in the laboratory, and that each board should carry sufficient creosote for the performance of standard tests.

Evaporation losses were in the order of volatility of the creosotes, as would be expected. The leaching losses are also in the same order, thus a creosote which has low permanence in a dry situation has also low permanence in water. In these experiments the losses of any given creosote by leaching were much greater than the losses by evaporation. The greater leaching losses of low boiling creosote no doubt result from the greater solubility of the lower boiling compounds, e.g., phenol is more soluble than cresols and cresols more than xylenols, and so on. Nevertheless, the loss of creosote C during the first three months was considerable and yet no significant change in chemical composition was noted. This loss may have been mechanical, analagous to the mechanical loss which occurred during the laboratory leaching of creosotes. This type of loss occurred with all creosotes, since the total loss was much greater than was accounted for by loss of tar acids.

The results of the shell and core tests show clearly the greater loss by leaching from the outer layers of treated timber. That loss of neutral oil as well as loss of tar acids occurred even from the core is shown by the following analysis. According to the analyses given for creosote A in (ii) above, in the board as treated, 100 g. of oven-dry wood was associated with 46·4 g. of neutral oil and 12·3 g. of tar acids; after leaching for two years 100 g. of oven-dry wood was associated with: in the shell, 19·3 g. of neutral oil and 1·3 g. of tar acids; in the core, 31·3 g. of neutral oil and 3·9 g. of tar acids. Since in the laboratory leaching tests the distillation range of the neutral oil was not materially affected, it appears that loss of neutral oil was not by solution but by mechanical loss of creosote as a whole. Furthermore, since loss of neutral oil occurred from the core, movement of creosote in the wood must have taken place.

Loss of creosote from a 1-in. layer of treated sapwood on a pole or pile would probably be less than one-half of the losses found in these tests, for, in the treated pile, only one face of the treated wood is exposed. In all these tests both faces and the edges of the test boards were exposed.

One week of leaching in the laboratory apparatus described above had an effect upon the creosote very similar to the effect of three years of leaching of the impregnated test board. The effects, viz., decrease in tar acid content and toxicity, were greater in the laboratory test. The relatively small drop in tar acid content resulting from a second week of leaching demonstrates the existence of a residue of tar acids of very limited solubility. It is to this fraction of the tar acids which vertical retort creosotes owe their permanence of toxicity, since most of the low boiling highly toxic tar acids are rapidly lost by leaching.

The decrease in the tar acid content of the creosotes in the evaporation specimens is obviously quite a different matter from the decrease in the leaching specimens where it depends upon solubility. The "unaccounted for creosote" appears to be related to this. By reference to Table 3 it may be seen that the decrease in tar acids was of the same order as the "unaccounted for creosote" for each one of the three oils, about 5 per cent. for A, 3 per cent. for B, and negligible for C. Thus it appears that the decrease in tar acids and the occurrence of unaccounted for creosote are both due to the deposition in the wood of a benzene-insoluble residue derived from the tar acids. Presumably it arises as an oxidation product of the tar acids.

The changes in S.G. of the creosotes correlate with the other changes noted and therefore give useful confirmation of these observations. The two principal changes, loss of the more volatile fractions and loss of tar acids, would have opposite effects on the S.G. of the oil as a whole. Loss of volatiles would cause the S.G. of the residual creosote to increase. Loss of tar acids, which are of higher S.G. than the neutral oil, would cause a decrease in the S.G. of the residual creosote. The initial increase of the S.G. of the light oil is in keeping with the loss of volatiles during treatment. The progressive decrease in its S.G. caused by leaching is in agreement with the loss of tar acids. The fact that S.G. of the heavy creosote remained constant during the three years of leaching confirms that the loss of this creosote was mainly mechanical. The variations in the S.G. of creosote A in the evaporation specimens may be accounted for by the loss of tar acids over the first six months, accompanied by steady losses of the more volatile fractions. The initial decrease caused by the former is now being outweighed by the increase caused by the latter. In the case of the two heavier creosotes the loss of tar acids by oxidation has not been sufficient to outweigh the steady increase due to loss of volatiles.

The results of these tests indicate that the test boards will retain effective concentrations of creosotes B and C for many years to come since the rate of loss is decreasing. This conclusion does not appear to be warranted for creosote A.

7. Acknowledgments.

This work was carried out at the Division of Forest Products under a grant from the Tar Distillers Research Association as part of an investigation into the properties of Australian vertical retort creosote oils. The entire examination of the test boards after three years of exposure was carried out by Mr. A. G. Charles of the Division of Forest Products during the absence of the author.

The Evaluation of D.D.T. as a Fungicide.

*By D. O. Norris, M.Sc. (Agric.)**

The new insecticidal compound D.D.T. (dichlorodiphenyltrichlorethane was tested for possible fungicidal properties. Preliminary trials were made in the laboratory with petri dish cultures in standard dishes 10 cm. by 2 cm. and using standard potato-dextrose agar.

Trial 1.—Twenty plates were prepared and divided into two lots. One set of plates was treated with 1 per cent. D.D.T. in alcohol by placing four drops of the solution on the agar at equal distances around the perimeter. The drops spread over an area about half an inch in diameter. The control plates were treated with drops of pure alcohol. All plates were incubated for 24 hours to evaporate the alcohol, and then seeded at the centre with the test fungi. Two plates each of treated and control lots were seeded with the following fungi:—*Ophiobolus graminis* Sacc., *Colletotrichum trifolii* Bain and Essary, *Pleospora herbarum* Pers., *Ascochyta imperfecta* Peck., and *Pseudoplea trifolii* (Rostr.) Petr. All plates were incubated at 25°C.

Twelve days later all fungi had grown impartially over all the plates, with no sign of retardation by D.D.T.

Trial 2.—Twenty-one plates were prepared in seven lots of three. To each plate was added 5 cc. of alcohol containing various concentration of D.D.T. The treatments of the different lots were as follows:—

Lot 1. Untreated.

Lot 2. 5 cc. of pure alcohol per plate.

Lot 3. 5 cc. of 1 per cent. D.D.T. (50 mg. D.D.T.) per plate.

Lot 4. 5 cc. of 0·1 per cent. D.D.T. (5 mg. D.D.T.) per plate

Lot 5. 5 cc. of 0·01 per cent. D.D.T. (0·5 mg. D.D.T.) per plate.

Lot 6. 5 cc. of 0·001 per cent. D.D.T. (0·05 mg. D.D.T.) per plate.

Lot 7. 5 cc. of 0·0001 per cent. D.D.T. (0·005 mg. D.D.T.) per plate.

The plates were incubated for one week to evaporate the alcohol, rewetted with sterile water, and seeded with the fungus *Ascochyta imperfecta*. Three weeks later after incubation at 25°C. all plates bore vigorous colonies. The two largest colonies were on plates containing 50 mg. of D.D.T.

Trial 3.—Finely-ground D.D.T. was made up in kaolin at 0·1, 1·0, 5·0, and 50·0 per cent. Lots of 200 seeds of commercial Greenfeast pea were dusted at the standard rate of 2 oz. per bushel with Spergon and with the D.D.T.–kaolin dusts. A control lot was left undusted. Soil was obtained that was known from experience to be heavily infested with unspecified damping-off organisms. This was uniformly

* An officer of the Division of Plant Industry.

mixed and placed in flats, one flat per treatment. The seed was sown in rows and lightly covered. Seven days later the seed in all treatments had swelled and pushed up the soil but, in all except the Spergon-treated flat, development went no further. After nine days the rows of seed in all the D.D.T. and the untreated control flats were clearly visible by virtue of a white mycelial mat on the surface of the soil. After fourteen days the following emergence figures were obtained:

Spergon	86·5 per cent.
D.D.T.–50 per cent.	0·0 per cent.
D.D.T.– 5 per cent. ..	0·0 per cent.
D.D.T.– 1 per cent. ..	1·0 per cent.
D.D.T.– 0·1 per cent. ..	1·0 per cent.
Control	0·0 per cent.

The experiment was a striking demonstration of the protective value of Spergon, but D.D.T. had no fungicidal value.

Trial 4.—Wheat of var. Free Gallipoli was heavily dusted with spores of flag smut (*Urocystis tritici* Koern). D.D.T. was made up as dusts in kieselguhr. Separate lots of smutted wheat were shaken up with prepared dusts until the wheat would not take up any more dust.

Each treatment was sown in five pots, with approximately 40 seeds per pot. After 50 days the plants were 9–10 inches high and the results as judged by the appearance of flag smut were obvious. The plants were therefore pulled up to facilitate observation of symptoms. The following percentages of infection were recorded for the different treatments:

Treatment.	Percentage of Infection.
Control—no fungicide	23
Control—Ceresan at standard rate of 2 oz. per bushel ..	0
D.D.T.–pure	33
D.D.T.–50 per cent.	24
D.D.T.–5 per cent.	17
D.D.T.–0·5 per cent.	31

D.D.T. had no effect in preventing infection.

The results of all trials showed that, with the range of organisms used, D.D.T. was of no value whatever as a fungicide. It may possess some value against other organisms not tested. A hint of this is contained in a report by Granovsky (*Amer. Potato J.* **24**: 89–90, April, 1944), in which the observation was made that potato plots dusted with D.D.T. dusts appeared to be somewhat less affected by leaf-parasitic fungi. It is highly improbable, however, that a substance which gave no indication of fungicidal properties in the above tests would possess any useful degree of toxicity when used in dust or spray form.

The Moisture Content of Meat Extract.

1. The Nature of Moisture Content.

*By Arthur R. Riddle, A.B., M.S.**

Summary.

The methods employed in industry for the determination of the moisture content of meat extract are briefly discussed, and evidence adduced to show that they are all likely to give differential values on the same extract both between themselves, and even between different determinations using any one method but varying the temperature and time. A discussion of the nature of water content follows, in which it is shown that the water liberated is probably due to (i) "free" water, (ii) so-called "bound" water, and possibly (iii) protein breakdown; that no dividing line exists between (i) and (ii); and that the curve of derived moisture as a function of time is continuous.

Since determinations of moisture content are purely arbitrary, and a true and unique value for it consequently not attainable, the need for a universally accepted and standardized method for its determination for all purposes of buying, selling, and inspection is indicated.

1. Introduction.

The estimation of water content in meat extract probably constitutes the greatest single difficulty with which the manufacturer of this product has to contend, a difficulty which is likely to persist until it is realized that, in the light of present knowledge, true water content of meat extract, or probably of any other foodstuff, is a quantity not capable of precise determination. To appreciate fully the reasons for this, a fundamental approach to the nature of moisture content is necessary.

Assays of moisture content of extract are mostly made by one or other of several variants of three main processes, namely:—

(i) Drying in an oven with or without moving air, at temperatures usually around 100°C., either for a definite period such as six hours, or to so-called constant weight, no provision being made for control of pressure.

(ii) Drying in a vacuum oven at various temperature levels.

(iii) Distillation with an immiscible liquid such as toluene, xylene, or petroleum derivatives.

A method based upon the amount of acetylene evolved by treating the quantitatively diluted extract with calcium carbide would probably be impracticable industrially.

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For the dispersal of the extract in oven-drying methods, vacuum or otherwise, the extract is usually diluted and transferred to metal boxes, preferably aluminium, containing asbestos, sand, pumice, or small glass beads, previously dried to constant weight at approximately 100°C. A method which has given very consistent results in this laboratory employs strips of blotting paper about 17½ in. by 1½ in., rolled up and placed in drying bottles and dried to constant weight. In toluene or similar distillation methods, the chief variant is the method of introduction of the sample of extract into the immiscible liquid employed, i.e., its state of division.

The dilution procedure adopted in this laboratory in oven-drying work is to dilute 10 g. of extract to make 100 ml. of solution. For drying, four 10-ml. aliquots of each diluted sample are employed. Before weighing out the extract, the sample is well mixed, this procedure being accomplished speedily to avoid as far as possible any interchange of moisture between the extract and the air with which it is in contact.

The curves obtained by plotting, in the case of distillation in an immiscible liquid, evolved water as a percentage of original sample weight or, in the case of oven-drying, percentage loss in weight, as functions of time, always show a steep initial rise before flattening out, as will be apparent in all the figures accompanying this article.

2. Experimental.

The experimental work from which curves *A* and *B* of Fig. 1 were plotted needs little explanation. The same well-mixed extract was used for both the toluene distillation run, where the temperature was approximately 110·5°C., and that with the oven where the extract, diluted as

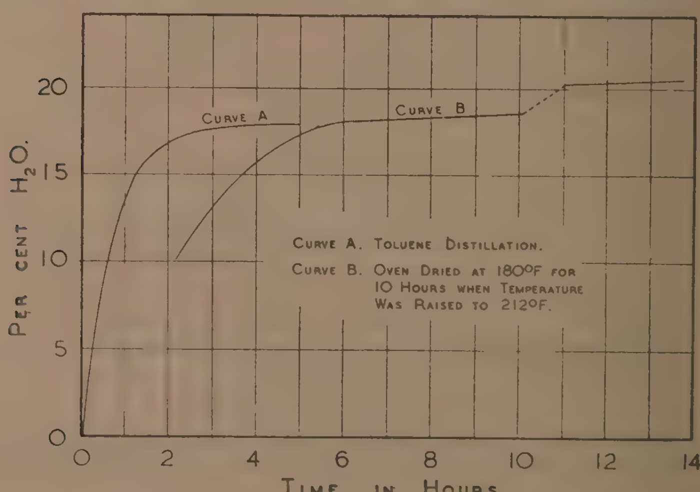

Fig. 1.—Percentage H_2O in meat extract as a function of time. Curve A, toluene distillation. Curve B, oven-dried at 82·2°C. for ten hours, when the temperature was raised to 100°C.

indicated above, was dispered on rolls of blotting paper and dried in moving air at $82 \cdot 2°C$. for ten hours, after which the temperature was raised to $100°C$., air temperatures being held to within $\pm 0 \cdot 1°C$.

An extensive series of experiments designed to secure information respecting (i) drying to constant weight, (ii) the influence of the amount and the mode of dispersal of the extract on the percentage weight loss during drying in vacuum at room temperatures and in an oven at 100°C., and (iii) the relative weight losses obtained by drying in vacuum at room temperatures as against those from oven-drying at 100°C., were carried out. The vacuum-drying and the oven-drying experiments were each continued for 60 days. In the vacuum series, the extract was in the forms of (i) an undiluted mass, (ii) diluted extract, (iii) diluted extract dispersed on blotting paper, (iv) diluted extract dispersed on sand. In the oven-drying series, amounts of approximately 2, 4, 6, and 8 g. of extract were each diluted with 10 ml. of water and dispersed on blotting paper as indicated above. On account of the extensiveness of the data, both in time and otherwise, it is not practicable to present it graphically, nor is this necessary. Points pertinent to this discussion, however, will be mentioned in their appropriate places.

3. Influence of Method of Preparing the Sample.

For the purposes of this article, it is sufficient to state that work done in this laboratory indicates that for the same extract:

(i) The manner of its dispersal for drying causes marked variations in the percentage weight losses obtained.

(ii) The diluted samples having the lower concentrations of extract give the higher percentage weight losses. Thus, in the 60-day oven-drying experiment, briefly mentioned above, the weight losses sustained by drying the 2, 4, 6, and 8 g. samples for ten days were 23·4, 21·5, 18·8, and 18·3 per cent. respectively. These values came closer together as time of drying increased, but at the end of 60 days the extremes were still 3 per cent. apart. This is confirmed by much other work dealing with mode of dispersal. Apparently the difficulty of removing water increases with increasing density of the diluted extract to be evaporated. This definitely suggests the formation of a hard surface skin of highly concentrated extract, the thickness of which increases with increasing concentration of the material to be evaporated, giving a "case-hardened" effect which retards evaporation. A similar effect is well known in many other forms of drying.

Following the breaking up of the material after thirteen days, all curves showed a definite upward trend indicating an acceleration in the rate of water removal, presumably due to the opening up of fresh evaporating surfaces.

In a further paper on the technique of drying, it is intended to present quantitative data on these and related matters.

4. Influence of Temperature.

Dedlow and Smith (1926), in describing a vacuum distillation method for the determination of moisture in meat extract, using xylene instead of toluene, incidentally show a set of curves obtained by the distillation method using naphtha distillates of various boiling points, reproduced here as Fig. 2. These, taken in conjunction with curves shown in Figs. 1 and 3, clearly indicate the influence of temperature in determining the amount of water evolved or, more correctly in the case of oven drying, the loss of weight on drying. In curve *B* in Fig. 1 the drying represented by the first part of the curve was done in moving air at 82·2°C. At the end of ten hours, the temperature was raised to 100°C., resulting in a quite abrupt change in the slope of the curve from the value commonly taken as constant weight.

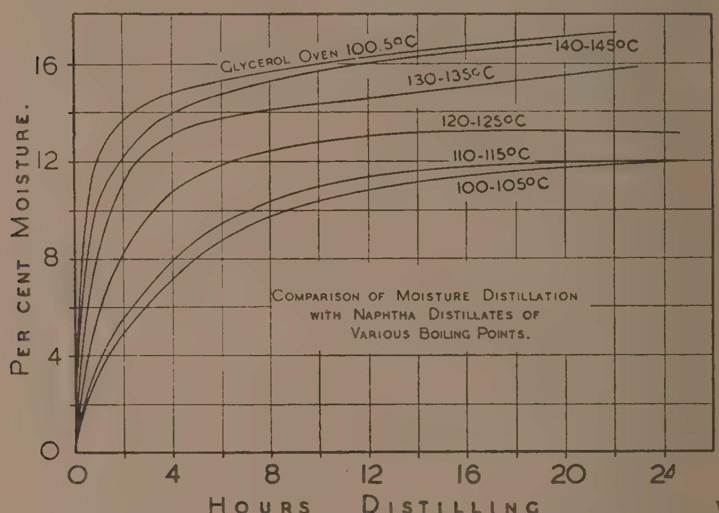

Fig. 2.—Percentage moisture in meat extract as a function of time of distilling with naphtha distillates of various boiling points. (Data of Dedlow and Smith.)

The results of considerable work done by the vacuum method at room temperatures—averaging 15°C. to 18°C.—over the period of drying, and by the ordinary oven method at 100°C., on the same extract, always show markedly higher values of weight loss in the case of oven-dried samples.

This influence of temperature on the evolution of moisture is also well demonstrated in the set of curves taken from a paper by Nelson and Hulett (1920) who worked extensively on the moisture content of coals and cereals, appearing as Fig. 3, showing moisture-time curves for an organic substance at varying temperatures.

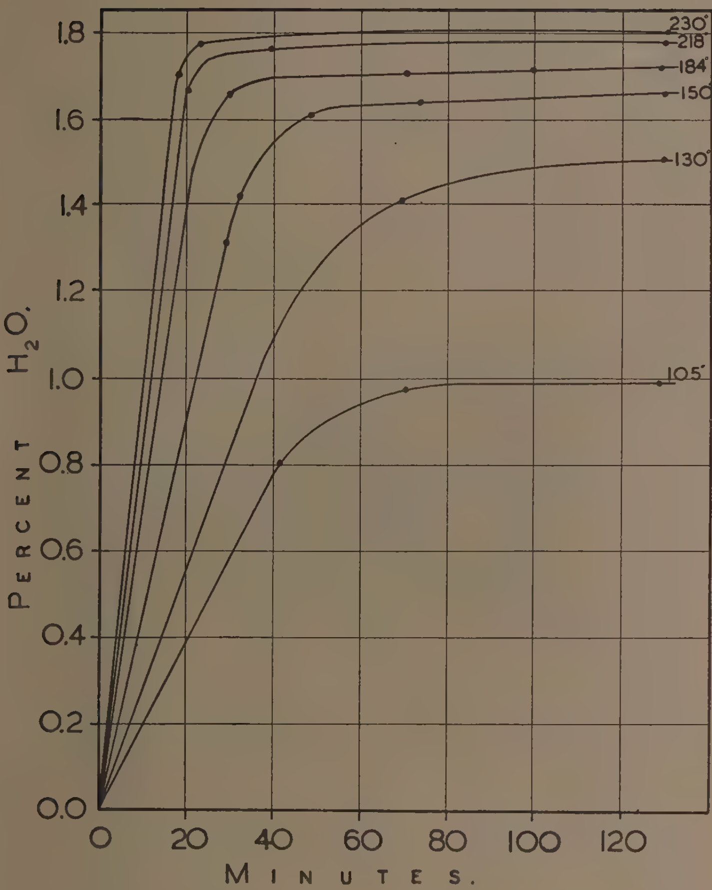

FIG. 3.—Curves of percentage H_2O derived from an organic material at various temperatures plotted as a function of time. (Data of Nelson and Hulett.)

5, Time of Drying and Constant Weight.

The usual instruction in most moisture-content work is to dry to constant weight. Because of doubts respecting the possibility of this in meat extract determinations, the two 60-day experiments mentioned above were made. While the vacuum samples did come to approximately constant weight, this did not occur until drying had gone on for roughly 40 days. In the oven-drying experiment at 100°C. constant weight was never entirely achieved, although the rate of weight loss became very small after approximately 30 days, being less than 0·5 per cent. per week.

6. Discussion.

(i) *"Free" and "bound" water.*—From the foregoing it is quite apparent that the value obtained for the moisture content of extract is a very variable one, depending chiefly on the method of preparation of the sample, the temperature of drying, and whether by oven methods or those involving distillation in an immiscible liquid. To appreciate the reason for this variability, it is necessary to realize that meat extract is a very complex substance, roughly 20 per cent. water, 20 per cent. mineral matter, and 60 per cent. organic matter. The latter contains protein material and, most important from an organoleptic point of view, the nitrogen bases creatin and its anhydride creatinin. In this organic material is much that is definitely colloidal in character. It may be postulated that in the course of its drying for moisture determinations, a condition will be attained ultimately where each colloidal "nucleus" is surrounded by a film of water only a comparatively few molecules thick. As this condition is approached, the strength of the forces binding the water to the colloidal nucleus, and thus the difficulty of water removal will increase rapidly, as reflected in a typical curve probably as the region where it turns over and tends to flatten out. Ultimately, a stage is reached where the water film around each particle has virtually no vapour pressure, and is very tenaciously adsorbed. This simplified picture neglects entirely the complex nature of both the colloidal and the crystalloidal material, and questions of solubility of the latter in the highly adsorbed water film, but does suggest roughly what may happen in one important phase of drying.

This adsorbed or "bound" water probably accounts for an appreciable portion of the total moisture content and may never be totally removed other than at very high temperatures. Gortner (1938) remarks, ". . . water is held with great tenacity upon surfaces, so that appreciable amounts of water may still remain in a colloid gel which has been heated to a rather high temperature." Rimington (1930), in connection with protein composition, says, "Certain considerations which are detailed below suggest that it is by no means improbable that proteins dried by the usual methods still contain an appreciable quantity of water held so tenaciously as to defy elimination by the ordinary means, and yet of sufficient quantity to vitiate analytical results" and later that " . . . it seems much more likely . . . that the discrepancy between analytical and calculated figures can be accounted for by quantities of 2 to 7 per cent. of water still adhering to the protein when constancy of weight in the drying process was attained." Nelson and Hulett (1920) concluded that water could not exist on surfaces at temperatures above that of its critical temperature of 365°C. Boswell and Dilworth (1925), however, in studies on the mechanism of catalysis by aluminium oxide, found that "There is still a water film on the surface, even after heating at atmospheric pressure at 500°C. for twenty hours, followed by two days' heating with a Meker burner."

This adsorption of water by hydrophilic colloids, characteristic of biological systems, is well described by Gortner (1938): "*The determination of the moisture content of a biological material is a purely empirical procedure determined by the three variables, temperature,*

pressure, and time. In order to make a definite statement that such and such a biological material has such and such a moisture content, it is necessary to define the conditions in regard to temperature, pressure, and time of drying under which the moisture determination was carried out. *The removal of water from a biocolloid is merely shifting one equilibrium between a colloid surface and water to a new equilibrium,* and the extent to which the equilibrium is shifted is determined by these three variables." This statement is well illustrated in curve *B* in Fig. 1. After approximately six hours' drying at 82·2°C., the rate of water loss had become very low, being roughly constant until the oven temperature was raised to 100°C. after the ten-hour samples had been removed from the oven. This disturbance of more or less equilibrium conditions was immediately followed by a rapid rise in weight loss, until a new approximation to equilibrium was established, as shown by the eleven-hour and subsequent samplings from which the curve is plotted.

(ii) *Protein breakdown.*—During the heating, at temperatures commonly employed in moisture determinations, breakdown of the protein in the extract probably occurs, giving rise to water and other substances. Nelson and Hulett (1920), who show gas-temperature curves for several vegetable substances, which gases of course occur only as a result of breakdown, state, "It is to be noted that there are no very sharp breaks in any of these curves, which is in accordance with the assumption that there are no definite temperatures of decomposition. However, when such a temperature is reached that appreciable amounts of water are liberated due to decomposition, the gas-temperature curves become relatively steep and in course of time almost parallel to the volume axis."

It is proposed to deal quantitatively with protein breakdown in meat extract in a later paper.

7. Conclusions.

It is probable that the water liberated in moisture content estimations at 100°C., and possibly at lower temperatures, includes (i) "free" water, (ii) "bound" water, and (iii) water produced by protein breakdown. In respect of the total of the first two, however, it must not be assumed that so much is "free" water, and so much "bound." There is, of course, no dividing line between the two. Further, at what temperature the water arising from protein breakdown becomes appreciable cannot at present be stated. No matter whence it comes, however, the water evolved is a continuous function of time, its rate changing, but no major discontinuity being apparent.

Certainly not at present, and probably never, will the attainment of a true and unique value for the moisture content of meat extract or, for that matter, of any biological product be possible—a value representing that due to so-called "free" and "bound" water only. Since any value is quite arbitrary, it is very desirable, therefore, that some one method, carefully defined and rigorously followed, be adopted by all those concerned with the industry, including Governmental agencies and representatives of both buyers and sellers. Admittedly the value obtained by any method adopted would be a purely arbitrary one, but

it would afford a definite standard which is all that is needed for commercial purposes. Whether or not the method adopted were an oven-drying one or one involving distillation in an immiscible liquid is immaterial, provided that the variables in the method selected could be so controlled as to give entirely consistent, though arbitrary, results on any one sample.

8. Acknowledgments.

Grateful acknowledgment is made of the generosity of the Queensland Meat Industry Board which, in addition to providing the laboratories and certain equipment, makes an annual contribution to the Council's Food Preservation and Transport Division's work in Brisbane; to Swift Australian Co. (Pty.) Ltd. for co-operation in placing at the Council's disposal manufacturing facilities without which this work could not have been accomplished; to Messrs. H. A. McDonald and H. J. E. Prebble for certain laboratory determinations; and to Miss J. E. O'Driscoll for draughting work.

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Tests for Quality in Egg Pulp.

2. Further Experiments on the Resazurin Reductase Test.

By W. J. Scott, B.Agr.Sc., and J. M. Gillespie, M.Sc.**

Summary.

Further experiments are described on the application of the resazurin reductase test to the quality grading of egg pulp. Data are given concerning the relationship between reduction time and bacterial plate count for pulps prepared in Victoria, New South Wales, Queensland, and South Australia. The regression equations calculated for these data show that there are no appreciable differences in magnitude between the equations for the various States. Experiments were also made to determine the optimal conditions of temperature for the performance of the test.

1. Introduction.

Following the experiments with 30 samples of Victorian egg pulp produced in 1942 (Scott and Gillespie, 1943) trials were continued in 1943. The investigations were extended to include samples of commercial pulp produced in New South Wales, Queensland, and South Australia, in order to determine whether the results found previously in Victoria applied to Australian pulp generally. All samples were collected from commercial establishments preparing egg pulp either for drying or for freezing.

These pulp samples were prepared from eggs of widely different grades of quality. In some instances, fresh first-quality eggs were used, but more commonly the eggs had been in cold storage for some months before being broken. In a few instances the pulp was prepared from cracked and second-grade eggs which had not been held in cold store. Generally, hand cracking methods were employed, but some samples were from pulp prepared in mechanical pulping devices. The samples of frozen pulp were all manufactured in Victoria and had been frozen for periods varying from two days to twelve months.

2. Methods.

The various techniques used in the reductase tests and accompanying plate counts were the same as those described previously (Scott and Gillespie, 1943). The reductase test was again carried out at a temperature of 30°C. and the plate count incubation temperature was 25°C. Some tests were made, however, at other temperatures and some comparative trials were carried out on various samples of resazurin.

3. Results.

(a) All Samples.

Tests were made on a total of 279 samples of freshly prepared egg pulp, comprising 162 from Victoria, 57 from New South Wales, 44 from Queensland, and 16 from South Australia. In addition, 52 samples of

---

* An officer of the Division of Food Preservation and Transport.

frozen pulp were examined, making a total of 331. Each sample was representative of at least 120 lb. of pulp. Observation of the reduction times was not continued beyond eight hours. Thirty-six samples did not reduce the resazurin within this time, and these samples have not been included in the data used for the calculation of regression equations. Of the 16 South Australian pulps only 3 reduced resazurin within eight hours. They also have been excluded from the data used for the calculation of regression equations. In Fig. 1 are summarized the results for the 295 samples for which both reduction times and plate counts were available. The regression coefficient is highly significant (see Equation (7) Table 3).

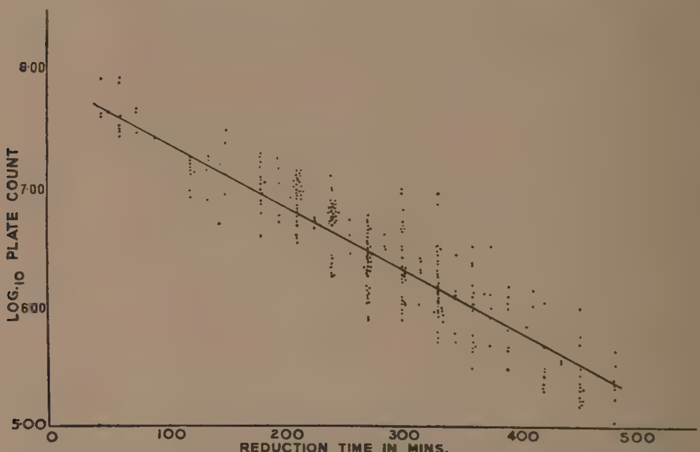

Fig. 1.—Resazurin reduction times and plate counts for 295 samples of Australian egg pulp.

The general relation between plate counts and reduction times is also shown for all samples in Tables 1 and 2 in which the range for one variable corresponding to each class in the other variable is given.

Table 1.—The Distribution of Plate Counts in Classes and the Range of Corresponding Reduction Times.

Number of Samples.	Plate Count per ml. (as logarithms).	Range of Reduction Times in Minutes.
11	< 3·99	> 480
24	4·00–4·99	> 480
70	5·00–5·99	180–480
174	6·00–6·99	105–420
52	> 7·00	45–240

TABLE 2.—THE DISTRIBUTION OF REDUCTION TIMES IN CLASSES AND THE RANGE OF CORRESPONDING PLATE COUNTS.

Number of Samples.	Reduction Times in Minutes.	Range of Plate Counts per ml. (as logarithms).
11	$\leq$ 60	7·60–7·91
17	$>$ 60 $\leq$ 120	6·54–7·90
23	$>$ 120 $\leq$ 180	6·90–7·49
69	$>$ 180 $\leq$ 240	5·84–7·30
68	$>$ 240 $\leq$ 300	5·88–7·11
56	$>$ 300 $\leq$ 360	5·70–6·99
26	$>$ 360 $\leq$ 420	5·48–6·50
25	$>$ 420 $\leq$ 480	5·16–6·04
36	$>$ 480	3·40–5·64

It will be seen from these tables and Fig. 1 that, although there is a definite relation between the logarithm of the plate count and the reduction time, individual samples do not always conform closely to the general regression equation.

(*b*) *Data for Samples from Various Centres of Production.*

The regression equations and other statistical data obtained in various centres of production are given in Table 3.

TABLE 3.—PLATE COUNT AND REDUCTION TIME REGRESSION EQUATIONS FROM VARIOUS CENTRES OF EGG PULP PRODUCTION.

Centre of Production.	Number of Samples.	Equation Number.	Regression Equation. $Y = a - b\,(x - \bar{x})$.	s.	Standard Error of Regression Co-efficient.
Vic. 1942 ..	30	1	$Y = 6{\cdot}48 - 0{\cdot}006092\,(x - 236{\cdot}5)$	0·138	0·000237
Vic. 1942–43	23	2	$Y = 5{\cdot}85 - 0{\cdot}005485\,(x - 330)$	0·122	0·000305
Vic. 1943–44	89	3	$Y = 6{\cdot}63 - 0{\cdot}006116\,(x - 269{\cdot}8)$	0·198	0·000231
Qld. 1943 ..	41	4	$Y = 6{\cdot}46 - 0{\cdot}003973\,(x - 304{\cdot}6)$	0·140	0·000253
N.S.W. 1943	57	5	$Y = 6{\cdot}49 - 0{\cdot}005286\,(x - 278{\cdot}95)$	0·154	0·000222
Vic. (Frozen) 1943	52	6	$Y = 6{\cdot}30 - 0{\cdot}005301\,(x - 251{\cdot}35)$	0·280	0·000326
Total ..	292	7	$Y = 6{\cdot}43 - 0{\cdot}005361\,(x - 275{\cdot}90)$	0·273	0·000158

Y = Log $_{10}$ plate count per ml.
x = Reduction time in minutes.

These regression lines are plotted in Fig. 2. It will be seen from Fig. 2 and Table 3 that the Queensland data give a line with a marked difference in the slope from the others. This difference is highly significant, but it is not certain if this is characteristic of all pulps produced in that area, as these samples were obtained largely from second quality and cracked eggs. The only other difference in the coefficients which approaches significance ($P=0 \cdot 05$) is the Victorian 1943-44 figure which is slightly greater than the coefficient for the combined samples.

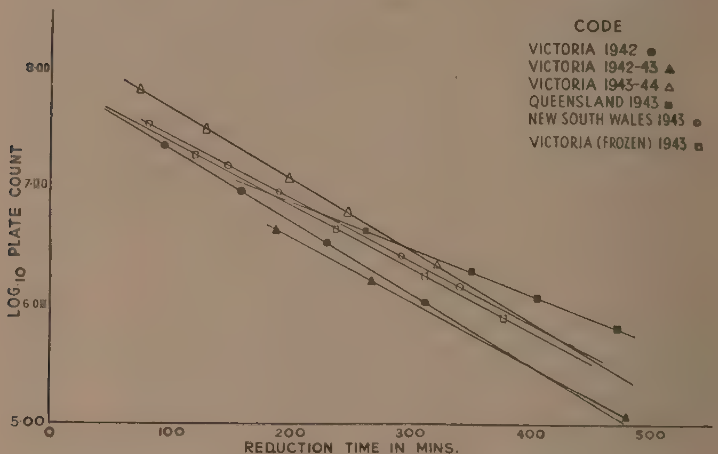

Fig. 2.—Resazurin reduction time and plate count regression equations for various centres of egg pulp production.

The lines in Fig. 2, which are approximately parallel, are separated by distances representing appreciable differences in plate count and most of these differences in position are statistically significant.

(c) *The Use of Various Samples of Resazurin.*

The following types of resazurin were found to behave similarly when tested; Eastman Kodak, B.D.H. Eastman Kodak, National Aniline and Chemical Co., Benger's tablets, and a sample synthesized according to the method of Baker *et al.* (1942). The lactose used in Benger's tablets was found to exert no measurable influence on reduction times. Samples marketed by Merck, Gurr, and British Drug Houses were found to be unsatisfactory for the purposes of this test. All the results reported in this paper were obtained with the Eastman Kodak reagent.

(d) *The Effect of Different Reduction and Plate Count Incubation Temperatures.*

The suitability of temperatures, other than those originally proposed, was tested on 20 samples of pulp. The results are given in Table 4.

TABLE 4.—THE INFLUENCE OF TEMPERATURE ON THE PLATE COUNT AND REDUCTION TIMES.

Plate Counts (Logarithmic Mean).		Reduction Times (Arithmetic Mean).	
25°C.	37°C.	30°C.	37°C.
990,000	76,400	279 mins.	360 mins.

The mean of the plate counts carried out at 37°C. was significantly lower ($P = 0 \cdot 01$) than those carried out at 25°C. and the mean of the reduction times at 37°C. was significantly longer ($P = 0 \cdot 02$) than that carried out at 30°C. In a number of trials the temperature of the reduction bath was maintained at 25°C., at which temperature there was a slight increase in reduction time which was not significant.

Summarizing these data it is obvious that plate count incubation at 25°C. gives substantially higher estimates than does incubation at 37°C. and no increase in the rate of dye reduction can be obtained by using temperatures slightly above or below 30°C. It appears therefore that there is no reason to depart from the temperatures proposed for both plate count and reduction determinations.

4. Discussion.

Amongst its other attributes, egg pulp should be the product of sound eggs and relatively free from contaminating bacteria. It is possible commercially to produce egg pulp with bacterial counts of less than 10,000 organisms per ml., but this can only be done when the eggs used are of excellent internal quality, and the method of cracking does not contribute contamination from shells and equipment.

The sanitation of a pulping plant is a complex matter, as there are generally numerous points at which bacterial contaminants can gain entrance into the pulp. The first source of contamination is the few faulty eggs, with high bacterial counts but no obvious signs of spoilage, which escape detection during the candling and subsequent cracking operations, and are emulsified with the main body of the pulp. Thus the addition of 1 per cent. of doubtful eggs with a count of ten million organisms per ml. could result in the contamination of sterile pulp with 100,000 organisms per ml.

The second source of contamination lies in the faulty hygiene of operatives. Cracking utensils and hands become readily soiled with bacteria-laden dirt from the shells and with contaminated material from cracked and rejected unsound eggs. Unless care is taken to enforce strict hygiene, serious contamination can occur.

Thirdly, egg pulp deposited in metal pipes, storage vats, and pumps is difficult to remove unless suitable detergents and methods are used. Any pulp remaining furnishes an excellent culture medium for bacteria,

and the resultant focus or foci of infection can provide serious contamination. For example, at a drying plant samples were taken at various stages in the production line from the point where the eggs were cracked and emulsified, through the storage vats to the spray jets. The plate counts and corresponding reduction times are shown in Table 5. The most serious contamination was acquired between the storage vats and the spray jets, and the reductase test provided this information within eight hours. Smaller increases in contamination, such as occurred prior to entry into the storage vats, were not detectable by the reductase test, and dependence on viable counts is necessary to discover such sources of contamination.

Table 5.—The Plate Count and Reduction Times of Pulp Samples Taken at Various Points on the Production Line.

Location of Sampling.	Plate Counts at 25°C. (organism per ml.).		Reduction Times (mins.)	
	Test 1.	Test 2.	Test 1.	Test 2.
From Pulper	5,000	9,600	> 480	> 480
Entrance to Vats	24,000	62,000	> 480	> 480
From Spray Jets	1,300,000	220,000	360	480

Although some highly significant differences exist in the slope and position of the various regression lines shown in Fig. 2, the differences generally are fairly small and may not be constant. It seems reasonable, therefore, to accept the general regression curve shown in Fig. 1 as a measure of the relationship between plate counts and reduction times for egg pulp produced in the various Australian States. As the relationship is highly significant the determination of reduction times may be applied to the bacteriological grading of egg pulp, for both freshly prepared and frozen products. The data presented were, however, obtained largely on samples with bacterial populations exceeding 100,000 per ml., and the results may be expected to apply only to products contaminated in excess of this level.

When using the test in industry, observation of the tubes during a period of eight hours will be necessary, and it would probably be convenient to transfer samples as collected to 0°C., and maintain them at that temperature overnight. Tubes prepared the following morning could then conveniently be observed for eight hours during the period of a working day. At least three quality grades could readily be defined. Samples failing to reduce the dye in eight hours could be considered of first quality, and two additional grades could be made depending on whether the colour change occurred within some shorter arbitrary time interval such as three hours.

5. References.

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NOTES.

Colonial Research Fellowships.

As one way of encouraging scientists to give special attention to problems of colonial interest, the British Government has instituted a number of " Colonial Research Fellowships " which will be open to graduates in the natural or social sciences who are under 35 years of age. The Fellowships will normally be for two years and provision has been made for 25 Fellowships within the next five years. The scheme will come into force immediately.

Candidates will be expected to provide evidence of their research experience, and their ability to plan and prosecute investigations of a high quality without close and constant supervision. The Fellowships will carry a basic allowance of £400 per annum, which may be increased to £600 per annum if the Fellow is married, or in other appropriate circumstances. Travelling expenses and a grant for apparatus will be provided, and employer's superannuation contributions will be met where necessary.

The Fellowship will be tenable in any part of the British Colonial Empire, and, where practicable, Fellows will be attached to centres of higher education in the Colonies. The awards will be conditional upon the candidate being certified as medically fit for the type of work to be undertaken.

Applications stating age, nationality, educational history, occupational history, and experience of research, should be addressed to the Secretary, Colonial Research Committee, Colonial Office, Downing-street, London, S.W.1., and they should indicate the precise nature of the problem on which the candidate wishes to do research. They should bear the endorsement of the head of the research institute to which the candidate is attached. Additional information may be obtained from the Secretary, C.S.I.R., 314 Albert-street, East Melbourne, C.2.

Buffalo Fly Investigations.

The attack on the buffalo fly is being pursued with vigour and as rapidly as possible, compatible with thoroughness. At its initial meeting recently, the newly appointed Buffalo Fly Committee consisting of representatives of the Council and the Queensland Department of Agriculture and Stock, exhaustively reviewed the situation in regard to this pest in Queensland.

The fly, which entered the State in 1928 at its extreme north-western corner where it adjoins the Northern Territory, did not spread for a number of years. Two factors were responsible for this. One was the restrictions, irksome perhaps to the stock owners at times but very wisely imposed by the State authorities, placed upon the movement of cattle from the infested area, and the other, the inability of the fly for some years to surmount the climatic and geographical barriers which hindered in infiltration to the east. In 1939, however, owing to the unusually favourable seasonal conditions then prevailing, an extension eastwards and southwards commenced, and this movement has continued notwithstanding the application of all possible measures that could in any way delay the spread of the pest. A careful consideration of the whole problem indicates that prevention of further spread southwards is clearly out of the question.

Ever since the fly entered the State serious attention has been given to it by scientific authorities. Its life history and habits were closely examined. Its potentialities for spreading were appreciated and, given suitable conditions, its ability to traverse certain natural barriers was quickly recognized.

Research work concerning control measures has been in progress for some considerable time and these are being vigorously prosecuted. One result of immediate practical application is the demonstration that, by means of a trap, largely based upon the horn fly trap of the United States of America, the fly population can, in small areas at least and where animals are under control as on dairy farms, be quickly reduced to almost negligible proportions. A pamphlet covering plans and specifications of the trap and its method of use is now in the press and should shortly be available for distribution.

Another line of investigation has embraced the application of sprays, particularly with the drug known as D.D.T., until recently on the secret list. Experiments have given very encouraging results but certain difficulties have yet to be overcome before it is possible to prepare a spray suitable for general use under field conditions. This aspect of the problem is being pressed as quickly as possible.

Reviews.

"BIBLIOGRAPHY ON INSECT PEST RESISTANCE IN PLANTS.

(Published by the Imperial Bureau of Plant Breeding and Genetics, Cambridge, England, 1944. pp. 40. Price 1s. 6d. Obtainable from the Central Sales Branch, Imperial Agricultural Bureaux, Agricultural Research Building, Penglais, Aberystwyth, Wales.)

The selection of plants for their resistance to damage by insects and other pests advances more and more into the foreground of any programme for the improvement of agricultural crops and other economic plants. The present bibliography provides an up-to-date survey covering the results of earlier work and the more recent findings of research into the morphological and physiological bases of such resistances. It includes a supplement on resistance to nematodes.

"MINERALS IN PASTURE—DEFICIENCIES AND EXCESSES IN RELATION TO ANIMAL HEALTH," by F. C. Russell, B.Sc.

(Technical Communication No. 15 of the Imperial Bureau of Animal Nutrition, Rowett Institute, Bucksburn, Aberdeen, Scotland, 1944, pp. 92. Price 5s. Obtainable from the Central Sales Branch, Imperial Agricultural Bureaux, Agricultural Research Building, Penglais, Aberystwyth, Wales.)

The occurrence of diseases such as enzootic marasmus and "coast disease" affecting sheep and cattle, and enzootic ataxia affecting sheep in both South and Western Australia has focused attention in Australia on the problems of mineral deficiencies in pastures. These diseases have been traced to the lack of either copper or cobalt or both in the pastures in the affected areas. Research has also been carried out on similar deficiencies in other countries, and there is now a great volume of literature on the subject. Apart from diseases caused by lack of certain minerals, ill-effects may also result from the presence of an excess of such toxic minerals as selenium and molybdenum.

This Technical Communication gives a comprehensive review of work in these fields.

Recent Publications of the Council.

Since the last issue of this *Journal,* the following publications of the Council have been issued:—

Bulletin No. 177.—"A Soil Map of Australia", by J. A. Prescott, D.Sc., A.A.C.I.

A tentative soil map of Australia was first published in 1930, and again in 1931. The map now presented on a scale of 1 to 10,000,000 is the result of a re-interpretation of data previously considered and many new personal observations made in the course of traverses made for this purpose in many parts of Australia. Eighteen soil groups or formations are shown in the map, and a brief description of each is given. Fuller discussion of the soil zones and their climatic, geological, and vegetational relationships has been left for a more extended study.

Bulletin No. 179.—"Lubrication between the Piston Rings and Cylinder Wall of a Running Engine," by J. S. Courtney-Pratt, B.E., and G. K. Tudor, B.E.

In the past, most of our information on this subject has been derived from measurements of the wear of the cylinder and the rings after a relatively protracted period of running and comparatively little has been known of what was happening while the engine was running. The present report describes an investigation into the detailed processes which occur during the sliding of the piston ring over the cylinder wall, and gives information of these processes even during the course of a single stroke.

The experimental method consists of an analysis, by a cathode ray technique, of the electrical resistance across the oil film between the moving piston rings and the cylinder wall while the engine is running. If the lubrication is complete and there is no metallic contact

through the oil film, the resistance is extremely high. When break-down of the lubricant film occurs and metallic contact occurs between the piston rings and cylinder wall, the resistance is very much lower. The electrical resistance at any instant is thus a measure of the amount of break-down of the lubricant film.

The results show that metallic contact between the cylinder wall and the piston rings can never be completely eliminated. Even using lubricating oils much heavier than are common in automobile practice intermittent breakdown of the lubricant film occurs. This effect is, in general, more marked in the regions of top and bottom dead centres and probably accounts for the increased wear in those portions of the cylinder. The results also show, as is to be expected, that increasing the engine speed or the viscosity of the oil reduces the amount of lubricant breakdown and metallic contact. The most marked effect, however, is that due to temperature. Any increase in the temperature of the oil film on the cylinder wall, leads to a marked increase in the amount of metallic contact. This deterioration in lubrication is much greater than can be accounted for simply by the decrease in viscosity due to the temperature rise.

These observations are of interest both from the fundamental and practical points of view. The results also demonstrate the applicability of this method of analysis to problems of cylinder lubrication and abrasive wear, its chief value being its analytical nature and the rapidity with which the results may be obtained as compared with conventional wear tests.

Forthcoming Publications of the Council.

At the present time, the following future publications of the Council are in the press:—

Bulletin No. 180.—" Studies on Deglutition in Sheep. 1.—Observations on the Course Taken by Liquids through the Stomach of the Sheep at Various Ages from Birth to Maturity," by R. H. Watson, D.Agr.Sc. " 2.—Observations on the Influence of Copper Salts on the Course Taken by Liquids into the Stomach of the Sheep," by R. H. Watson, D.Agr.Sc., and I. G. Jarrett, B.Sc.

Bulletin No. 181.—" Sheep Blowfly Investigations. The Attractiveness of Sheep for *Lucilia cuprina*," by I. M. Mackerras, M.B., Ch.M., B.Sc., and M. J. Mackerras, M.B., M.Sc.

Bulletin No. 182.—" The Effectiveness of Various Mineral Dusts for the Control of Grain Pests," by J. S. Fitzgerald, M.Sc., Ph.D., A.A.C.I.

Bulletin No. 183.—" Experimental Determination of the Influence of the Red-legged Earth Mite (*Halotydeus destructor*) on a Subterranean Clover Pasture in Western Australia," by K. R. Norris, M.Sc.

Bulletin No. .—" Fellmongering Investigations," by F. G. Lennox, D.Sc., Margaret E. Maxwell, M.Sc., and W. J. Ellis, A.S.T.C.

Bulletin No. .—" Studies on the Mitchell Grass Pasture in South-Western Queensland. 2.—The Effect of Grazing on the Mitchell Grass Pasture," by R. Roe, B.Sc. (Agric.), and G. H. Allen, Dip. Agric. (Lawes).

PLATE 1.

Stock and Scion Investigations. (See page 221.)

Twig of Delicious showing roughly circular cracked areas in bark.

PLATE 2.

Stock and Scion Investigations. (See page 221.)

Twig of Jonathan showing "pimpled" bark. The bark has been pared from the lower portion of the twigs showing dead brown-black areas in the wood below the "pimples."

PLATE 3.

Stock and Scion Investigations. (See page 221.)

Twig of Delicious showing terminal die-back and confluent bark cracks of the twig on the left. The lower shoot on the right is apparently healthy.

PLATE 4.

Stock and Scion Investigations. (See page 221.)

Portion of shoot of Jonathan showing lesion healing over after borax treatment.

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INDEX TO VOLUME 17.

PAGE.

Analysis—

Mechanical, of Soils, Use of Hydrometer for 197

Quantitative Spectrochemical, of Agricultural Samples 225

Anderson, A. J.—

Automatic Irrigator Actuated by a Soil-Moisture Tensiometer .. 151

Double-Compartment Pot Cultures for Studies in Plant Nutrition .. 144

Apple Rootstock Investigations—

Apple Measles 221

Root-Systems of Some Own-rooted Apple Trees 167

Austin, C. R., and Shipton, J.—Determination of Carotene 115

Bald, J. G.—

Development of Differences in Yield between FX and Virus X-infected Up-to-date Potatoes 263

Ground Work Studies on Growth of Normal Potato Foliage .. 94

Progress of Work with Potato Stocks free from Virus X (FX Potatoes) 258

Bald, J. G., and Helson, G. A. H.—Estimation of Damage to Potato Foliage by Potato Moth 30

Barrie, N.—*See* Shaw, N. H.

Beetroot, Canned, *Cl. botulinum* in 16

Bland, D. E.—Permanence of Australian Vertical Retort Creosote Oils .. 274

Blowfly Dressings—*See* Sheep Blowfly Dressings.

Boas, I. H., Retirement from Division of Forest Products 130

Boron Deficiency Diseases—Apple Measles 221

Botulism—Influence of Tin on Growth of *Cl. botulinum* 16

Buffalo Fly Investigations 305

Bull, L. B., and Mules, M. W.—Investigation of *Myxomatosis cuniculi* with Special Reference to the Possible Use of the Disease to Control Rabbit Populations in Australia 79

Carbon Bisulphide for Bag-Stack Fumigation of Wheat 208

Carotene, Determination of 115

Carrots, Canned, *Cl. botulinum* in 16

Centrifugation in Fractionation of Colloidal Material 23

Ceresan—

Effect on Field Emergence and Yield of Garden Peas 71

Evaluation of D.D.T. as a Fungicide in Comparison with 289

INDEX—*continued*.

PAGE.

Clostridium botulinum and *Cl. sporogenes*, Influence of Tin on Growth of .. 16

Clover, Subterranean—

Dormancy and Hardseededness 186, 191, 242, 251, 255

Reaction to Attack by *Uromyces trifolii* as a Heritable Character .. 74

Colloids—Fractionation with Supercentrifuge 23

Colonial Research Fellowships 305

Connectors, Timber—*See* Timber Connectors.

Copper-Lead Alloys—Preparation of Sections for Metallographic Examination 130

Couchman, J. F.—Effect of Straw Maturity on the Chemical Composition of Flax Straw and Fibre 139

Creosote Oils, Australian Vertical Retort, Permanence of 274

Cryolite, Synthetic, for Control of Potato Moth 179

Cryptostegia grandiflora, Rubber from 49

Cuprox—Effect on Field Emergence and Yield of Garden Peas 71

D.D.T.—

as a Fungicide 289

in Control of Buffalo Fly 305

Downes, R. G.—Use of the Hydrometer for the Mechanical Analysis of Soils 197

Dressings, Sheep Blowfly 1

Egg Pulp, Resazurin Reductase Test for Quality in 299

Ethylene Chlorohydrin, Production of 213

Fellowships, Colonial Research 305

Fertilizers—

Effect of Lime, Phosphate, and Molybdenum on Growth of Lucerne .. 233

Nitrogenous, Effect on Yield of Garden Peas 69

Fisheries Investigations—Live-bait Fishing for Tuna 59

Flax Straw and Fibre, Effect of Straw Maturity on Chemical Composition of 139

Flett, A.—Report on Live-bait Fishing for Tuna in Australia 59

Foodstuffs, Stored, Effect of Diurnal Temperature Fluctuations on . 111

Fungi, Dictionary of—Review 130

Fungicidal Dusts—

Effect on Field Emergence and Yield of Garden Peas 71

Evaluation of D.D.T. 289

INDEX—*continued.*

PAGE.

Gillespie, J. M.—*See* Scott, W. J.

Gnorimoschema operculella—*See* Potato Moth.

Guayule, Rubber from 49

Helson, G. A. H.—Preliminary Field Test of Insecticides against Potato Moth 179

See also Bald, J. G.

Hicks, E. W.—Estimation of Effect of Diurnal Temperature Fluctuations on Reaction Rates in Stored Foodstuffs and Other Materials .. 111

Hills, K. L.—

Dormancy and Hardseededness in *T. subterraneum* 186, 191, 242, 251, 255

Reactions of Varieties of *T. subterraneum* to Attack by *Uromyces trifolii* as a Heritable Character 74

Hosking, J. S.—Use of the Sharples Supercentrifuge in the Fractionation of Colloidal Material 23

Hutton, E. M.—

Effect of Nitrogenous Manures on the Yield of Garden Peas .. 69

Field Emergence and Yield of Garden Peas as Affected by Treatment with Fungicidal Dusts 71

Hydrometer for the Mechanical Analysis of Soils 197

India—Council of Scientific and Industrial Research 65

Insecticides for Control of Potato Moth—*See* Potato Moth.

Irrigator, Automatic, Actuated by a Soil-Moisture Tensiometer .. 151

Kipps, E. H.—*See* Shaw, N. H.

Kloot, N. H.—Tests on the Holding Power of Titan Plain and Processed Cement-coated Nails 156

Kok-saghyz, Rubber from 49

Leaf Area of Potatoes—*See* Potato Foliage.

Leaf Rust of Clover—*See Uromyces trifolii.*

Lightfoot, G., Retirement from Secretaryship 207

Lime, Effect of, on Growth of Lucerne 233

Live-bait Fishing for Tuna 59

Lucerne, Effect of Lime, Phosphate, and Molybdenum on Growth of .. 233

INDEX—*continued.*

PAGE.

McTaggart, Alexander—Obituary 207

Manures—*See* Fertilizers.

Meat Extract, Moisture Content of 291

Mice in Bag Stacks of Wheat, Control of 208

Moisture Content—

of Meat Extract 291

of Timber used as a Partition between Water and Air 133

Molybdenum, Effect of, on Growth of Lucerne 233

Mules, M. W.—*See* Bull, L. B.

Murray, K. E.—Ethylene Chlorohydrin: Laboratory Investigation of a Continuous Process for its Production on a Commercial Scale .. 213

Myxomatosis 79

Nails, Tests on Holding Power of 156

Norris, D. O.—Evaluation of D.D.T. as a Fungicide 289

Obituary—Dr. Alexander McTaggart 207

Oertel, A. C.—Quantitative Spectrochemical Analysis of Agricultural Samples 225

Pear Diseases—Apple Measles 221

Peas, Garden—

Effect of Nitrogenous Manures on Yield 69

Field Emergence and Yield as Affected by Fungicidal Dusts .. 71

Phenothiazine for Control of Potato Moth 179

Phosphate, Effect of, on Growth of Lucerne 233

Photographic Printing, Automatic Timing of Exposures of 127

Plant Nutrition Studies, Pot Cultures for—*See* Pot Cultures.

Pot Cultures, Double-Compartment—

Automatic Irrigator for 151

Use in Plant Nutrition Studies 144

Potato Foliage—

Estimation of Damage by Potato Moth 30

Ground Work Studies on Normal Growth 94

Potato Moth—

Estimation of Damage to Potato Foliage by 30

Field Test of Insecticides against 179

INDEX—*continued.*

PAGE.

Potato Virus Diseases—

Development of Differences in Yield between FX and Virus X-infected Up-to-Date Potatoes 263

Ground Work Studies on Growth of Normal Potato Foliage .. 94

Progress of Work with Potato Stocks free from Virus X (FX Potatoes) 258

Publications of the Council—

Forthcoming 68, 132, 211, 308

Recent 66, 131, 211, 307

Rabbit Myxomatosis 79

Resazurin Reductase Test for Quality in Egg Pulp 299

Research, Fundamental Scientific 208

Research Fellowships, Colonial 305

Reviews—

"Bibliography on Insect Pest Resistance in Plants" 306

"Dictionary of Fungi" 130

"Minerals in Pasture—Deficiencies and Excesses in Relation to Animal Health" 307

Riddle, A. R.—Moisture Content of Meat Extract 291

Roberts, H. D.—Moisture Content Distribution in Wood used as a Partition between Water and Air 133

Rootstock Investigations—*See* Apple Rootstock Investigations.

Rubber from Plant Sources 49

Rubber, Synthetic—Note on Australian Investigations 49

Scott, W. J., and Gillespie, J. M.—Tests for Quality in Egg Pulp .. 299

Scott, W. J., and Stewart, D. F.—Influence of Dissolved Tin on the Growth of *Clostridium botulinum* in Canned Vegetables 16

Seed, Subterranean Clover—*See* Clover, Subterranean.

Shaw, N. H., Barrie, N., and Kipps, E. H.—Effect of Lime, Phosphate, and Molybdenum on the Growth of Lucerne in Duntroon Loam .. 233

Sheep Blowfly Dressings B.T.B. and B.K.B. 1

Shipton, J.—*See* Austin C. R.

Soil Analysis—

Mechanical Use of Hydrometer for 197

Quantitative Spectrochemical 225

Soil Colloids—Fractionation with Supercentrifuge 23

Soil-Moisture Tensiometer for Actuating an Automatic Irrigator .. 151

INDEX—*continued.*

PAGE.

Spectrochemical Analysis, Quantitative, of Agricultural Samples .. 225

Spergon—

Effect on Field Emergence and Yield of Garden Peas 71

Evaluation of D.D.T. as a Fungicide in Comparison with 289

Split-ring Connectors—*See* Timber Connectors.

Sprays, Borax, for Apple Measles 221

Stewart, D. F.—*See* Scott, W. J.

Subterranean Clover—*See* Clover, Subterranean.

Supercentrifuge, Sharples 23

Temperature Fluctuations, Diurnal—Effect on Reaction Rates in Stored Foodstuffs 111

Tensiometer, Soil-Moisture—*See* Soil-Moisture Tensiometer.

Thomas, L. A.—Stock and Scion Investigatons—

IV. Apple Measles 221

III. Root-systems of some Own-rooted Apple Trees 167

Timber Connectors, Split-ring, Protection against Corrosion of 162

Timber Preservation—Permanence of Australian Vertical Retort Creosote Oils 274

Timber used as a Partition between Water and Air, Moisture Content Distribution in 133

Timing, Automatic, of Exposures of Photographic Printing 127

Tin, Dissolved, Influence on *Clostridium botulinum* 16

Townsend, A. A.—Automatic Timing of Exposures of Photographic Printing 127

Trifolium subterraneum—*See* Clover, Subterranean.

Tuna, Live-bait Fishing for 59

Uromyces trifolii, Reaction of Subterranean Clover to Attack by .. 74

Vegetables, Canned, *Cl. botulinum* in 16

Vitamin A—Determination of Carotene 115

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